

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
12 October 2000 (12.10.2000)

PCT

(10) International Publication Number
WO 00/59532 A1(51) International Patent Classification⁷: A61K 38/39

(21) International Application Number: PCT/US00/08678

(22) International Filing Date: 31 March 2000 (31.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/127,391 1 April 1999 (01.04.1999) US(71) Applicant (for all designated States except US): BIOS-
TRATUM, INC. [US/US]; Suite 120, 2605 Meridian Park-
way, Durham, NC 27713 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): BROOKS, Peter
[US/US]; 7054 Hawthorn Avenue, Hollywood, CA 90028
(US). HUDSON, Billy [US/US]; 15414 West 94th Street,
Lenexa, KS 66219 (US).(74) Agent: HARPER, David, S.; McDonnell, Boehnen, Hul-
bert [en:amp] Berghoff, Suite 3200, 300 South Wacker
Drive, Chicago, IL 60606 (US).(81) Designated States (national): AE, AL, AM, AT, AU, AZ,
BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK,
DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL,
IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,
LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT,
RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TZ, UA,
UG, US, UZ, VN, YU, ZA, ZW.(84) Designated States (regional): ARIPO patent (GH, GM,
KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent
(AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent
(AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU,
MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM,
GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

with international search report

(48) Date of publication of this corrected version:

15 November 2001

(15) Information about Correction:

see PCT Gazette No. 46/2001 of 15 November 2001, Sec-
tion IIFor two-letter codes and other abbreviations, refer to the "Guid-
ance Notes on Codes and Abbreviations" appearing at the begin-
ning of each regular issue of the PCT Gazette.

(54) Title: THE USE OF DOMAINS OF TYPE IV COLLAGEN T INHIBIT ANGIOGENESIS AN TUMOUR GROWTH

(57) Abstract: The instant invention provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and en-
dothelial cell interactions with the extracellular matrix, involving contacting the tumor, animal tissue, or endothelial cells with an
amount effective to inhibit angiogenesis, tumor growth and metastasis, or endothelial cell interactions with the extracellular matrix
of an antagonist of specific integrin receptors.

WO 00/59532 A1

THE USE OF DOMAINS OF TYPE IV COLLAGEN T INHIBIT ANGIOGENESIS AND TUMOUR GROWTH

5

Cross Reference

This application claims priority to U.S. Provisional Application Serial No. 60/127,391 filed April 1, 1999, and is a continuation in part of U.S. Application Serial No. 09/277,665, filed March 26, 1999, which is a continuation in part of U.S. Application Serial No. 09/183,548 filed October 30, 1998, which is a continuation of 10 08/800,965 filed February 18, 1997, now U.S. Patent No. 5,856,184.

Field of the Invention

This invention relates to methods and kits for inhibiting angiogenesis, tumor 15 growth and metastasis, and endothelial cell interactions with the extracellular matrix.

Background of the Invention

Angiogenesis, the process of formation of new blood vessels, plays an important role in physiological processes such as embryonic and postnatal 20 development, as well as in wound repair. Formation of blood vessels can also be induced by pathological processes involving inflammation (e.g., diabetic retinopathy and arthritis) or neoplasia (e.g., cancer) (Folkman, 1985, *Perspect. Biol. Med.*, 29, 10). Neovascularization is regulated by angiogenic growth factors secreted by tumor or normal cells as well as the composition of the extracellular matrix and by the activity of 25 endothelial enzymes (Nicosia and Ottinetti, 1990, *Lab. Invest.*, 63, 115).

During the initial stages of angiogenesis, endothelial cell sprouts appear through gaps in the basement membrane of pre-existing blood vessels (Nicosia and Ottinetti,

1990, *supra*; Schoefl, 1963, Virehous Arch, Pathol. Anat. 337, 97-141; Ausprunk and Folkman, 1977, Microvasc. Res. 14, 53-65; Paku and Paweletz, 1991, Lab. Invest. 63, 334-346). As new vessels form, their basement membrane undergoes complex structural and compositional changes that are believed to affect the angiogenic response

5 (Nicosia, et. al., 1994, Exp. Biology, 164, 197-206). Early planar culture models have shown that basement membrane molecules modulate the attachment, migration, proliferation, and organizational behavior of endothelial cells (Nicosia, et. al., 1994, *supra*). More recent studies with three-dimensional aortic culture models that more closely simulate angiogenic conditions during wound healing in vivo suggest that the

10 basement membrane is a dynamic regulator of angiogenesis, and its function varies according to its molecular components (Nicosia, 1994, *supra*).

A common feature of all solid tumor growth is the requirement for a blood supply. Therefore, numerous laboratories have focused on developing anti-angiogenic compounds based on growth factors and their receptors. While this approach has led to

15 some success, the number of growth factors known to play a role in angiogenesis is large. Therefore, the possibility exists that growth factor antagonists may have only limited use in treating cancer since tumors and associated inflammatory cells likely produce a wide variety of factors that can induce angiogenesis.

In this regard, a strategy that targets a common feature of angiogenesis, such as

20 endothelial cell adhesion to the extracellular matrix (ECM), might be expected to have a profound physiological impact on tumor growth in humans. This notion is supported by the fact that RGD-containing antagonists of the $\alpha v \beta 3$ integrin ECM cell adhesion receptor can block angiogenesis. (U.S. Patent No. 5,766,591) Furthermore, the $\alpha v \beta 3$ integrin is expressed most prominently on cytokine -activated endothelial and smooth

muscle cells and has been shown to be required for angiogenesis. (Vamier et al., Cell Adhesion and Communication 3:367-374 (1995); Brooks et al., Science 264:569-571 (1994)). Based on these findings, a potentially powerful new approach to anti-angiogenic therapy might be to specifically target critical regulatory domains within
5 distinct ECM components.

The basement membrane (basal lamina) is a sheet-like extracellular matrix (ECM), which is a basic component of all tissues. The basal lamina provides for the compartmentalization of tissues, and acts as a filter for substances traveling between tissue compartments. Typically the basal lamina is found closely associated with an
10 epithelium or endothelium in all tissues of an animal including blood vessels and capillaries. The basal lamina components are secreted by cells and then self assemble to form an intricate extra-cellular network. The formation of biologically active basal lamina is important to the development and differentiation of the associated cells.

Type IV collagen has been shown to be a major structural component of
15 basement membranes. The protomeric form of type IV collagen is formed as a heterotrimer made up from a number of different subunit chains called $\alpha 1(IV)$ through $\alpha 6(IV)$. Up to now, six genetically distinct α -chains belonging to two classes with extensive homology have been identified, and their relative abundance has been demonstrated to be tissue specific. The type IV collagen heterotrimer is characterized
20 by three distinct structural domains: the non-collagenous (NC1) domain at the carboxyl terminus; the triple helical collagenous domain in the middle region; and the 7S collagenous domain at the amino terminus. (Martin, et. al., 1988, Adv. Protein Chem. 39:1-50; Gunwar, et. al. 1991, J. Biol. Chem. 266:14088-14094).

The ability to express recombinant α (IV) NC1 domains provides the opportunity to study the effect of specific domains on many biological processes, such as angiogenesis, tumor metastasis, cell binding to basement membranes, and assembly of Type IV collagen molecules.

5

Summary of the Invention

The instant invention provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interaction with the extracellular matrix, each method comprising contacting the tumor, animal tissue, or endothelial cells with antagonists of specific integrin receptors.

10

Brief Description of the Drawings

Figure 1 illustrates the effects of NC1 (Hexamer) and 7S domains of Type IV collagen at a 50 μ g/ml concentration on angiogenesis from mouse thoracic aorta organ cultures.

15 **Figure 2** illustrates the effects of 7S domain of Type IV collagen on angiogenesis from mouse thoracic aorta organ cultures. The domain concentrations employed in this experiment were 0 μ g/ml (control); 0.5 μ g/ml; 5 μ g/ml and 50 μ g/ml.

Figure 3 illustrates the effects of NC1 (Hexamer) domain of Type IV collagen on angiogenesis from mouse thoracic aorta organ cultures. The domain concentrations employed in this experiment were 0 μ g/ml (control); 5 μ g/ml and 5 μ g/ml and 50 μ g/ml.

20

Figure 4 are photographs of mouse thoracic aorta segments embedded in Matrigel (EHS basement membrane matrix, Collaborative Biomedical Products, Bedford, MA) at 5 days of culture. Control specimen (0 μ g/ml of NC1 (Hexamer) and 7S domains)

exhibited growth of microvessels from the cultured tissue into the matrix (Figure 4A). In contrast, angiogenesis was inhibited in specimens cultured with 50 $\mu\text{g/ml}$ of 7S domain (Figure 4B) and NC1 (Hexamer) domain (Figure 4C).

Figure 5 is a graphical representation of data demonstrating the in vivo effect of IV
5 injection of recombinant ($\alpha 1$) type IV collagen monomer on angiogenesis using fibrin implants in rats.

Figure 6 is a graphical representation of data demonstrating that the recombinant ($\alpha 1$) and ($\alpha 2$) NC1 monomers inhibit the bFGF-induced increase in angiogenic index in vivo.

10 Figure 7 is a graphical representation of demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on the bFGF-induced increase in total blood vessel branch points in vivo.

Figure 8 is a graphical representation of data demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on the bFGF-induced increase in angiogenic index in
15 vivo.

Figure 9 is a graphical representation of data demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on the bFGF-induced increase in angiogenic index in vivo.

Figure 10 is a graphical representation of data demonstrating the effect of recombinant
20 ($\alpha 1$) and ($\alpha 2$) NC1 monomers on mean CS-1 melanoma tumor weight in vivo.

Figure 11 is a graphical representation of data demonstrating the dose response effect of recombinant ($\alpha 2$) NC1 monomer on mean CS-1 melanoma tumor weight in vivo.

Figure 12 is a graphical representation of data demonstrating the effect of recombinant ($\alpha 1$), ($\alpha 2$), and ($\alpha 4$) NC1 monomers on mean HT1080 tumor weight in vivo.

Figure 13 is a graphical representation of data demonstrating the effect of recombinant ($\alpha 1$), ($\alpha 2$), ($\alpha 3$) and ($\alpha 5$) NC1 monomers on mean HEP-3 tumor weight in vivo.

Figure 14 is a graphical representation of data demonstrating human endothelial cell adhesion to immobilized NC1 α monomers.

- 5 Figure 15 is a graphical representation of data demonstrating the effect of soluble $\alpha 1$ and $\alpha 2$ NC1 monomers on human endothelial cell adhesion to pepsinized collagen type IV.

Figure 16 is a graphical representation of data demonstrating the effect of isolated recombinant NC1 monomers on human endothelial cell migration in vitro.

- 10 Figure 17 A-F provides the sequences of each type IV collagen α chain monomer.

Figure 18 is a graphical representation of data demonstrating the effect of monoclonal antibodies against various integrins on human endothelial cell adhesion to recombinant the ($\alpha 2$) NC1 domain.

- 15 Figure 19 is a graphical representation of data demonstrating human endothelial cell adhesion to the recombinant ($\alpha 1$) NC1 domain.

Description of the Preferred Embodiments

- Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A*
20 *Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press),
Gene Expression Technology (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutscher, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press,

San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique*, 2nd Ed. (R.I. Freshney. 1987. Liss, Inc. New York, NY), and *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.).

As used herein, the term Type IV collagen domain encompasses the group of molecules including the non-collagenous NC1 domain (Hexamer) and 7S collagenous domains, as well as NC1 α chain monomers.

The invention comprises methods for using Type IV collagen NC1 α -monomers (ie: $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 6$), which are defined to include such monomers isolated from any multicellular organism or produced via recombinant protein expression from a gene encoding such a monomer from any multicellular organism, and also to encompass various modifications, additions, and/or deletions to such monomers.

In one aspect, the present invention provides methods and kits for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 6$ NC1 chain monomers.

In another aspect, the present invention provides methods and kits for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 6$ NC1 chain monomers.

In another aspect, the present invention provides methods and kits for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit metastasis of a polypeptide composition comprising one or more

isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 6$ NC1 chain monomers.

In a further aspect, the present invention provides methods and kits for inhibiting endothelial cell interactions with the extracellular matrix in tissue comprising
5 contacting the tumor or tissue with an amount effective to inhibit endothelial cell interactions with the extracellular matrix of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 6$ NC1 chain monomers.

The NC1-encoding domain of each of the six α chain cDNAs has been cloned
10 into a vector for recombinant protein expression as previously described (Sado et al., *Kidney Intl.* 53:664-671 (1998), incorporated by reference herein in its entirety). The vectors are used to stably transfect human kidney 293 cells, which produce the recombinant protein. The DNA and deduced amino acid sequences of the recombinant type IV collagen alpha chain monomers produced as described are shown in Figure
15 17A-F. The first 17 amino acids correspond to a BM40 signal sequence (which is cleaved from the mature protein), to facilitate protein secretion. All the secreted proteins (ie: mature proteins) start with the sequence APLA followed by the affinity tag, DYKDDDDK at the amino terminus. This tag facilitates purification and identification of the material, and does not interfere with biological activity of the
20 recombinant NC1 α chain monomers.

The type IV collagen NC1 α chain monomers can be produced by any method known in the art, including using recombinant DNA technology or biochemical peptide synthesis technology, or by isolating the NC1 domains from animal sources, such as from basement membrane sources such as bovine lens capsule and bovine kidney glomeruli.

(Peczon et al., Exp. Eye Res. 30:155-165 (1980); Langeveld et al., J. Biol. Chem. 263:10481-10488 (1988); Gunwar et al., J. Biol. Chem. 266:14088-14094 (1991))

In practicing the invention, the amount or dosage range of type IV collagen NC1 α chain monomers employed is one that effectively inhibits angiogenesis, tumor growth, tumor metastasis, and/or endothelial cell-extracellular matrix interactions. An inhibiting amount of NC1 α chain monomers that can be employed ranges generally between about 0.01 $\mu\text{g/kg}$ body weight and about 10 mg/kg body weight, preferably ranging between about 0.05 $\mu\text{g/kg}$ and about 5 mg/kg body weight.

The NC1 α chain monomers may be administered by any suitable route, including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intraarterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. In preferred embodiments, the NC1 α chain monomers are administered intravenously or subcutaneously.

The NC1 α chain monomers may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (e.g., solutions, suspensions, or emulsions). The NC1 α chain monomers of the invention may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the NC1 α chain monomers, and are not harmful for the proposed application.

The NC1 α chain monomers may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

For administration, the NC1 α chain monomers are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanolic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidone, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined by the claims appended hereto.

Example 1 – In Vitro Effect on Angiogenesis

With modifications, the procedures of Nicosia and Ottinetti (1990), *supra*, and Nicosia, et. al. (1994), *supra*, were utilized for experiments designed to test the effect of Type IV collagen on angiogenesis under *in vitro* conditions. The model has been used to study the effects of growth factors and extracellular matrix molecules on the

angiogenic response and employs aortic rings cultures in three-dimensional collagen gels under serum-free conditions. These experiments are outlined below.

A. Methods

Experiments were performed with 1-3 month old Swiss Webster male mice.

- 5 Following anesthesia, the thoracic aorta was excised under aseptic conditions and transferred to sterile MCDB 131 sterile growth medium (Clonetics, San Diego, CA) containing antibiotics. Fat was dissected away from the aorta and approximately six to eight 1 mm thoracic segments were obtained from each specimen. Segments were transferred to 48 well tissue culture plates. The wells of these plates were layered with
- 10 100 microliters of Matrigel (EHS basement membrane, Collaborative Biomedical Products, Bedford, MA) prior to transfer of the aortic segments. The Matrigel was diluted 1:1 with MCDB 131 growth medium prior to use. The segments were centered in the wells and an additional 100 microliters of Matrigel was then placed over the specimens. The aortic segments were therefore embedded in the basement membrane
- 15 matrix. Each well then received 300 microliters of MCDB 131 growth medium. The plates were placed in an incubator maintained at 37° C with 5% CO₂. Specimens were observed daily over a 7 day period. Newly growing microvessels were counted using an inverted phase microscope at various times during the culture period, but data is expressed at 3 and 5 days of culture. To test for the effect of Type IV collagen on
- 20 angiogenesis, domains at known concentrations were mixed with the Matrigel and with the MCDB 131 growth medium. Fresh MCDB 131 growth medium (plus and minus collagen domains) was changed every 3 days.

B. Results

After establishing the time course of angiogenesis under control conditions (Matrigel plus MCDB 131 growth medium), experiments were performed using various concentrations of Type IV collagen (isolated from bovine lens) NC1 (hexamer) and 7S domains. Data represents the analysis of at least 3 specimens per experimental condition. In the first experiment (**Figure 1**), analysis indicated that at a concentration of 50 µg/ml, NC1 domain and 7S domain significantly inhibited angiogenesis as monitored at 3 and 5 days of culture. In the second experiment, various concentrations of these domains were analyzed. As indicated in **Figure 3**, 7S domain at 50 µg/ml again significantly inhibited angiogenesis at 3 and 5 days. Inhibition was reduced at 5 and 0.5 µg/ml concentrations. As indicated in **Figure 2**, NC1 domain was less effective in blocking angiogenesis as compared to that observed in the first experiment (**Figure 1**), although it was still effective. In addition, as compared to the 7S domain, there was less of a correlation between concentration and inhibitory action.

Figure 4A-C are photographs of mouse thoracic aorta segments embedded in Matrigel (EHS basement membrane matrix, Collaborative Biomedical Products, Bedford, MA) at 5 days of culture in the presence or absence of 50 µg/ml of Type IV collagen domains. The control specimen (no domains) exhibited growth of microvessels from the cultured tissue into the matrix (**Figure 4A**). In contrast, angiogenesis inhibition was observed in tissues cultured in the presence of 50 µg/ml of 7S (**Figure 4B**) and NC1 (Hexamer) domain (**Figure 4C**).

Example 2. Subcutaneous fibrin implant angiogenesis

Recombinant human type IV collagen NC1 ($\alpha 3$) monomer (Sado et al., Kidney International 53:664-671 (1998)) was injected intravenously in Fisher 344 rats containing fibrin implants surgically placed subcutaneously, a modified version of the method described by Dvorak et al (Lab. Invest. 57(6):673-686 (1987)). The implants were then removed and directly analyzed using an inverted microscope. The analysis involved counting the number of blood vessels that had grown into the fibrin in the control and experimental group.

Briefly, 4 fibrin implants were surgically implanted subcutaneously into Fisher 344 rats (2 dorsal and 2 ventral sides). The average rat weight was approximately 125 grams.

Three rats (EXP) were given tail vein injections of either control (fibrin alone), 100 μ l of 100 μ g/ml of 7S domain of type IV collagen (approximately 0.80 mg/kg body weight), 100 μ l of 100 μ g/ml of type IV collagen hexamer (approximately 0.80 mg/kg body weight), or recombinant collagen type IV NC1 ($\alpha 3$) monomer at a concentration of 1.26 mg/ml in PBS (120 μ g protein, or approximately 0.96 mg/kg body weight) and 3 rats (C) were given 100 μ l tail vein injections of PBS. Injections of recombinant protein were given every other day for five doses. The injection schedule was as follows:

Day 1: (implant day) injection and remove blood sample (EXP and C)
Day 3: Injection (EXP and C)
Day 5: Injection and remove blood sample (EXP and C)
Day 7: Injection (EXP and C)
Day 9: Injection and remove blood sample (EXP and C)
Day 11: Remove and fix implants (save blood sample) (EXP and C)

The results of one experiment were as follows:

2 week in vivo experiment:

Control (fibrin alone)

about 66 BV

7S domain of type IV lens collagen (100 µg/ml)	None
Hexamer of type IV lens collagen (100 µg/ml)	None
Monomer ($\alpha 3$)	None

5 The results are shown as the mean number of blood vessels per implant. The results of this study demonstrate that isolated domains of type IV collagen, including the $\alpha 3$ monomer, can significantly inhibit capillary growth in the in vivo fibrin clot implant model. In subsequent experiments, the inhibitory effect was occasionally seen to attenuate with time, suggesting that higher dosages or more frequent injections might
10 be even more effective.

A similar experiment was conducted using recombinant human type IV collagen NC1 ($\alpha 1$) monomer (100 µl of a 1 µg/µl solution; approximately 0.80 mg/kg body weight) and comparing the number of blood vessels that had grown into the fibrin at day 11 of treatment relative to the control group. Three rats per group were analyzed
15 with each rat having 4 implants. These experiments demonstrated that administration of the $\alpha 1$ monomer significantly inhibited capillary growth in the in vivo fibrin clot implant model (Figure 5).

Example 3. Recombinant NC1 ($\alpha 2$) domain inhibits angiogenesis in vivo

20 We next tested the effects of systemic administration of soluble NC1 α -chain monomers in the chick embryo CAM angiogenesis assay.

Angiogenesis was induced in the CAMs of 10 day old chick embryos with bFGF as described (Brooks et al., Cell 92:391-400 (1998)). Twenty four hours later the embryos were systemically treated with various concentrations of recombinant NC1 α -
25 chain monomers, in a total volume of 100 µl of sterile phosphate buffered saline (PBS). Two days later the embryos were sacrificed and the filter discs and CAM tissues

removed. Angiogenesis was quantitated by counting the number of angiogenic blood vessel branch points in the confined area of the filter disc. The Angiogenic Index is defined as the number of branch points from experimental treatment minus control treatment.

5 In initial experiments, recombinant $\alpha 1$ or $\alpha 2$ NC1 domains were injected at a concentration of 50 μg per embryo. At this concentration, the NC1 domains were shown to be highly toxic as demonstrated by greater than 90% embryo cell death. However, at lower doses they were well tolerated and showed potent anti-angiogenic activity. A total of 6 individual angiogenesis experiments were conducted with the
10 NC1 domains. However, in two experiments, the bFGF induction was low, making it difficult to interpret the results. The NC1 $\alpha 2$ domain appeared to be more consistent and potent than the $\alpha 1$ NC1 domain at inhibiting angiogenesis. In fact, systemic administration of 30 μg of NC1 $\alpha 2$ consistently inhibited angiogenesis by greater than 90% (Figures 6-9), as measured by inhibition of the bFGF-induced increase in the
15 angiogenic index and the mean number of blood vessel branch points. In contrast, NC1 $\alpha 1$ domain showed variable inhibitory activity (0%-50%) throughout the experiments.

Example 4. Recombinant NC1 domain inhibits melanoma tumor growth in vivo:

20

Since the growth of all solid tumors depends on angiogenesis to provide nutrients for its continued expansion, reagents that have the capacity to inhibit angiogenesis may significantly inhibit tumor growth. Therefore, we tested the effects of recombinant NC1 domains of type IV collagen for their effects on tumor growth in
25 vivo.

To test the effects of NC1 domains on tumor growth in vivo, we utilized the chick embryo tumor growth assay. Briefly, single cell suspensions of 3 distinct tumor types were applied to the CAM of 10 day old chick embryos. The tumors included CS-1 Melanoma cells (5×10^6), HT1080 human fibrosarcoma cells (4×10^5) and Hep-3 human epidermoid carcinoma cells (2×10^5). The embryos were injected systemically with varying concentrations of NC1 α -chain monomers 24 hours later. The embryos were next allowed to incubate for a total of 7 days, at which time they were sacrificed. The resulting tumors were resected and wet weights determined. A total of 6 tumor growth assays were conducted with the 3 distinct tumor types. A single injection of 10 μ g NC1 $\alpha 2$ domain inhibited CS1 melanoma tumor growth by approximately 70% relative to control (Figure 10). In similar experiments, dose response curves were completed with CS-1 tumors. Systemic administration of NC1 $\alpha 2$ resulted in a dose-dependent inhibition of CS-1 melanoma tumor growth in vivo with a maximum inhibition following a single dose at 30 μ g (Figure 11). Systemic administration of NC1 $\alpha 1$ also inhibited CS-1 tumor growth but it was variable and in some experiments failed to inhibit tumor growth (See Figure 10). In similar experiments, NC1 $\alpha 2$ inhibited HT1080 human fibrosarcoma tumor growth by approximately 50% after a single systemic injection of 30 μ g, while NC1 $\alpha 1$ and $\alpha 4$ had no effect (Figure 12). Finally, systemic administration of NC1 $\alpha 2$ (30.0 μ g) and $\alpha 3$ inhibited Hep-3 human epidermoid carcinoma tumor growth by approximately 40% and 60% respectively, and $\alpha 1$ inhibited Hep-3 tumor growth by approximately 30%, while NC1 $\alpha 5$ domain failed to inhibit tumor growth (Figure 13).

We conclude from these in vivo studies that tumor growth can be inhibited by isolated NC1 α -chain monomers. These molecules can thus be used alone, or to

complement the use of existing anti-tumor agents, in providing enhanced and more effective anti-tumor therapy.

Example 5. Immobilized NC1 domains support human endothelial cell adhesion

5 In order for new blood vessels to form, endothelial cells must have the capacity to adhere and migrate through the ECM. Moreover, this endothelial cell-ECM interaction may facilitate signal transduction events required for new blood vessel formation. Therefore, since type IV-collagen is an ECM protein which is known to support cell adhesion, we tested the ability of the NC1 domains to support endothelial
10 cell attachment.

Microtiter plates were coated with 25 μ g/ml of purified NC1 domains followed by incubation with 1% bovine serum albumin (BSA) to block non-specific interactions. Human endothelial cells (ECV304) were then allowed to attach to the immobilized NC1 domains for 1 hour. Non-adherent cells were removed by washing and attached
15 cells were quantified by measuring the optical density (O.D.) of crystal violet eluted from attached cells. Data bars represent the mean +/- standard error of the O.D. from triplicate wells.

Immobilized NC1 α 2, α 3, and α 6 domains supported endothelial cell adhesion while NC1 α 1, α 4, and α 5 domains promoted little if any cell adhesion (**Figure 14**).
20 Soluble NC1 α 1 (a1) and α 2 (a2) inhibited endothelial cell adhesion to pepsinized collagen type IV by approximately 50% (**Figure 15**).

Taken together, these findings demonstrate that isolated, recombinant NC1 domains from the α 1, α 2, α 3, and α 6 chains of collagen type IV can mediate human endothelial cell adhesion and/or inhibit endothelial cell adhesion to ECM proteins in

vitro, and suggest that the potent anti-angiogenic and anti-tumor activity of the isolated NC1 domains is due to disruption of endothelial cell interaction with the extracellular matrix that are necessary for angiogenesis.

5 **Example 6. Endothelial Cell Migration**

Invasive cellular processes such as angiogenesis and tumor metastasis also require cellular motility. Thus we evaluated the ability of isolated NC1 domains to support human endothelial cell migration in vitro. These experiments were conducted essentially according to the methods in Brooks et al., J. Clin. Invest. 99:1390-1398
10 (1997).

The results of these experiments indicate that NC1 $\alpha 2$, $\alpha 3$, and $\alpha 6$ domains can support human endothelial cell migration in vitro, while $\alpha 1$, $\alpha 4$, and $\alpha 5$ domains showed little if any capacity to support endothelial cell migration (FIG 16).

15 **Example 7. Efficacy in Lewis lung in vivo tumor**

The above studies indicated that specific domains of collagen type IV can promote cell migration in vitro. Thus, we evaluated the ability of NC1 domains to support endothelial cell migration in vivo.

The α (IV) NC1 domain hexamer, isolated by enzymatic digestion of bovine
20 lens capsule basement membrane by known protocols (Peczon et al., Exp. Eye Res. 30:155-165 (1980)) was tested in the metastatic Lewis lung mouse tumor model using a standard protocol which is considered to be a good model of both metastasis and angiogenesis of lung tumors. (See for example, Teicher et al., Anticancer Res.

18:2567-2573 (1998); Guibaud et al., *Anticancer Drugs* 8:276-282 (1997); Anderson et al., *Cancer Res.* 56:715-718 (1996)).

Each study consisted of an untreated control group and six treatment groups. There were ten animals per treatment group with 40 mice in the control. In each study, all treatment was administered intravenously once every 2 days for 7 doses starting one day after tumor inoculation. Dosages of α (IV) NC1 hexamer were either 100 $\mu\text{g}/\text{mouse}$ or 200 $\mu\text{g}/\text{mouse}$. In the Lewis lung study, the tumor cell inoculum was 1×10^6 viable cells. All animals were weighed twice a week throughout the study. Starting one day after the last treatment, 5 mice were periodically sacrificed from each control group to measure pulmonary tumor burden. The experiment was terminated at day 14 when the lungs of the control animals had sufficient tumor mass to provide meaningful evaluation. At that time, the lungs of all remaining animals were excised, weighed, and the number of tumor foci greater than 2 mm in diameter counted. The resulting data showed that both dosages of α (IV) NC1 hexamer significantly reduced the number of visible lung metastases (Mann-Whitney Rank Sum Test, $p < 0.05$), with 8 visible lung metastases in the control, vs. 5 (100 $\mu\text{g}/\text{mouse}$) and 4 (200 $\mu\text{g}/\text{mouse}$), and the 100 $\mu\text{g}/\text{mouse}$ dosage reduced the lung weights from a median of 520 mg in controls to a median of 462 mg in experimental, while the median lung weight of mice treated with 200 $\mu\text{g}/\text{mouse}$ was 620 mg.

Other in vivo studies demonstrated that tumor cell metastasis to the lung can be reduced by 50% or more using intravenous injections of the Type IV collagen domains in murine B16 melanoma, human A375SM melanoma xenografts. Furthermore, injection of the NC1 hexamer also significantly reduced the number of lung tumors in separate Lewis Lung tumor studies.

Example 8. Defining the Integrin Receptor Mediating Cellular Adhesion to the NC1 domains

To define the integrin receptors that mediated cellular adhesion to the NC1 $\alpha 1$
5 and $\alpha 2$ domains, adhesion assays were performed as described in Example 5 in the
presence or absence of function blocking monoclonal antibodies directed to specific
integrins (Figures 18 ($\alpha 2$); Fig. 19 ($\alpha 1$)). These antibodies were directed against $\alpha 5\beta 3$
integrin (anti-avb3), the $\alpha 5\beta 5$ integrin (anti-avb5), the $\beta 1$ integrin ($\beta 1$) (all described in
U.S. Patent No. 5,766,591, incorporated by reference herein in its entirety), and
10 monoclonal antibodies directed against the $\alpha 1$ (anti-a1), $\alpha 2$ (anti-a2), and $\alpha 3$ (anti-a3)
integrins (purchased from Chemicon, California). These studies indicated that human
endothelial cells interact with NC1 $\alpha 2$ domain primarily through $\alpha v\beta 5$ and $\alpha v\beta 3$
integrins with variable contribution from $\beta 1$ integrins (Figure 18). In similar
experiments, anti- $\beta 1$ integrin antibodies showed a lesser effect on endothelial cell
15 adhesion to NC1 $\alpha 2$, suggesting a lesser contribution of $\beta 1$ integrins to this adhesive
activity. In contrast, endothelial cell adhesion promoted by NC1 $\alpha 1$ domain was
mediated by integrin $\alpha 3\beta 1$ (Figure 19).

Previous studies have demonstrated that RGD-containing antagonists of the
 $\alpha v\beta 3$ receptor can block angiogenesis (U.S. Patent No. 5,766,591), but the instant
20 invention provides the first demonstration of a non-RGD containing antagonist of the
 $\alpha v\beta 3$ integrin that can block angiogenesis. The present study also demonstrates that
antagonists of the $\alpha v\beta 5$ integrin and the $\alpha 3\beta 1$ integrins can block angiogenesis.

Thus, the instant invention also provides methods and kits for inhibiting
angiogenesis, tumor growth and metastasis, and endothelial cell interaction with the
25 extracellular matrix, each method comprising contacting the tumor, animal tissue, or

endothelial cells with antagonists of specific integrin receptors. Specifically, the methods comprise contacting the tumor, animal tissue, or endothelial cells with one or more of the following polypeptide compositions:

- (a) a polypeptide composition comprising one or more non-RGD containing
5 integrin $\alpha\beta 3$ antagonists; or
- (b) a polypeptide composition comprising one or more antagonists of $\alpha\beta 5$
integrin; or
- (c) a polypeptide composition comprising one or more antagonists of $\beta 1$
integrins; or
- 10 (d) a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$
integrins.

We conclude from all of the above studies that angiogenesis, tumor growth and metastasis, and endothelial cell adhesion to the ECM, can be inhibited by isolated,
15 recombinant domains of type IV collagen, or by antagonists of specific integrin receptors. The present invention is thus broadly applicable to a variety of uses which include inhibition of angiogenesis and treatment of diseases and conditions with accompanying undesired angiogenesis, such as solid and blood-borne tumors including but not limited to melanomas, carcinomas, sarcomas, rhabdomyosarcoma,
20 retinoblastoma., Ewing sarcoma, neuroblastoma, osteosarcoma, and leukemia.

The invention is further applicable to treating non-tumorigenic diseases and conditions with accompanying undesired angiogenesis, including but not limited to diabetic retinopathy, rheumatoid arthritis, retinal neovascularization, choroidal neovascularization, macular degeneration., corneal neovascularization, retinopathy of

prematurity., corneal graft rejection, neovascular glaucoma., retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sogrens, acne rosacea, phlyctenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, 5 bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi's sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, traum, systemic lupus, polyarteritis, Wegeners sarcoidosis, scleritis, Steven's Johnson disease, radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occlusion, carotid 10 obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular tissue, hemangiomas, Osler-Weber-Rendu, acquired immune deficiency syndrome, ocular neovascular disease, osteoarthritis, 15 chronic inflammation, Crohn's disease, ulcerative colitis, psoriasis., atherosclerosis, and pemphigoid. See U.S. Patent No. 5,712,291)

The invention is also broadly applicable to methods for inhibiting tumor growth and metastasis, reduction of scar tissue formation, reduction of complications due to cell adhesion in organ transplants, and the inhibition of lymphocyte adhesion and 20 mobility.

While the fundamental novel features of the invention have been shown and described, it will be understood that various omissions, substitutions, and changes in the form and details illustrated may be made by those skilled in the art without departing from the spirit of the invention. For example, various modifications,

additions, and/or substitutions can be made to the type IV collagen α monomer chains that would be encompassed by the invention.

We claim

1. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha\beta 3$ antagonists.
2. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\alpha\beta 5$ integrin.
3. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.
4. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.
5. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha\beta 3$ antagonists.
6. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\alpha\beta 5$ integrin.
7. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial

cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.

8. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

9. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.

10. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.

11. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.

12. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

13. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.

14. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.

15. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\alpha 3 \beta 1$ integrin.
16. A method for inhibiting tumor growth in tissue comprising contacting the tumor
5 or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

1/30

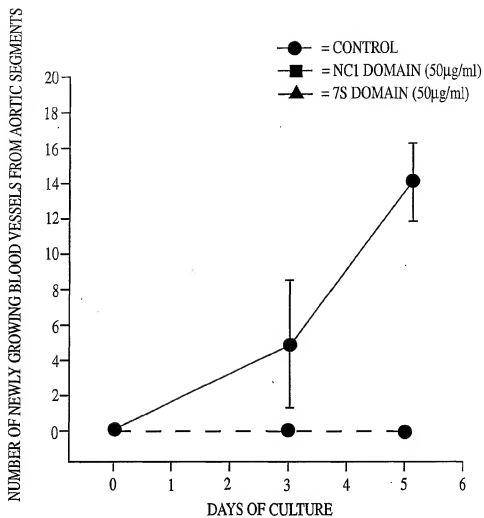


FIG. 1

2/30

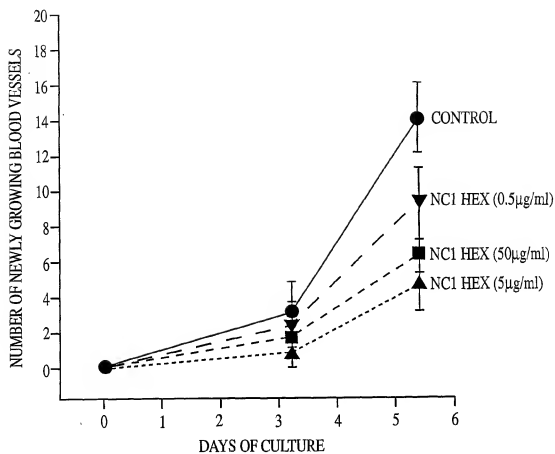


FIG. 2

3/30

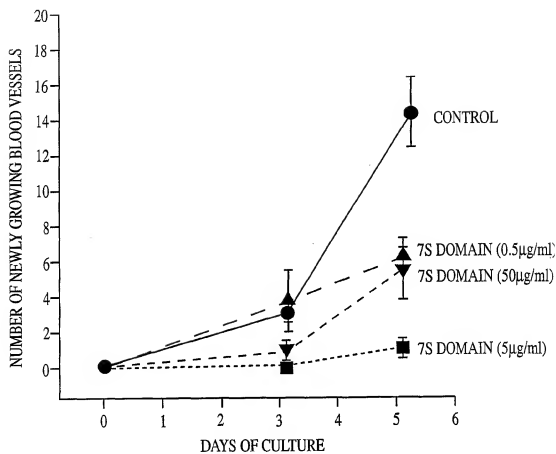


FIG. 3

4/30



CONTROL
FIG. 4a



7S DOMAIN (50 μ g/ml)

FIG. 4b



NC1 DOMAIN (50 μ g/ml)

FIG. 4c

5/30

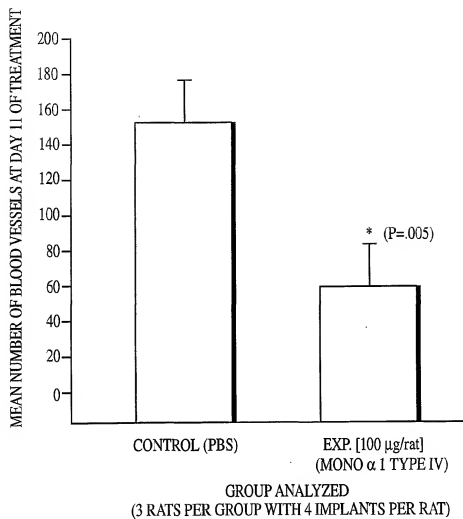


FIG. 5

6/30

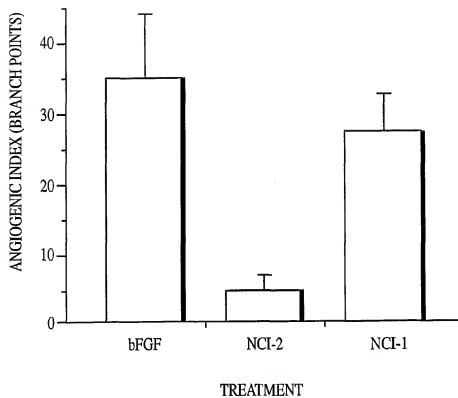


FIG. 6

7/30

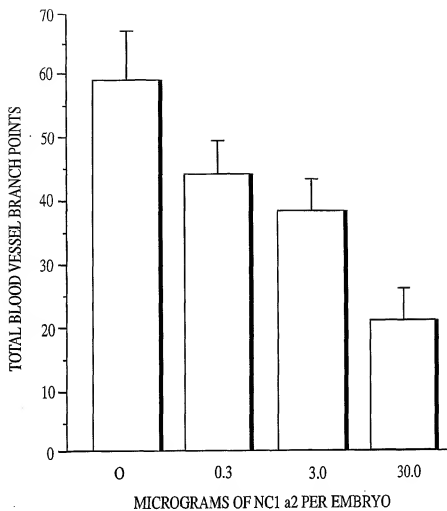


FIG. 7

8/30

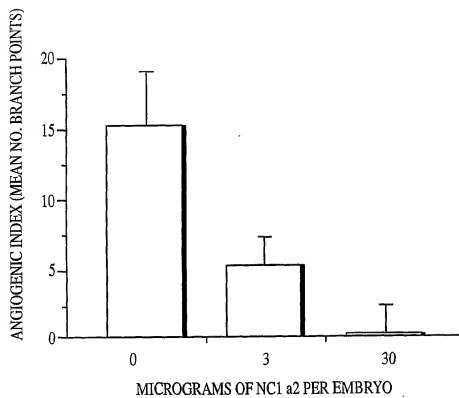


FIG. 8

9/30

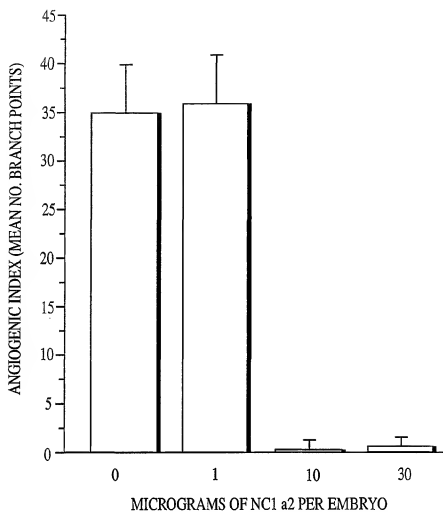


FIG. 9

10/30

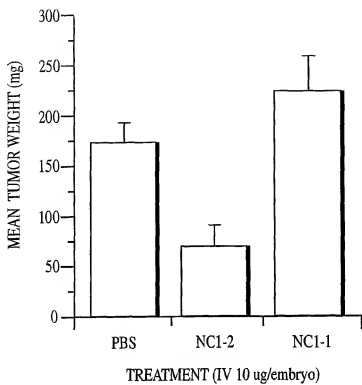


FIG. 10

11/30

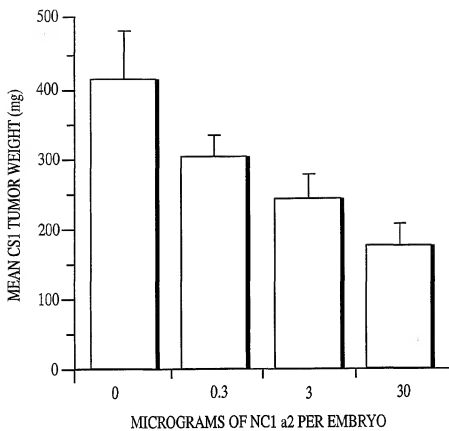


FIG. 11

12/30

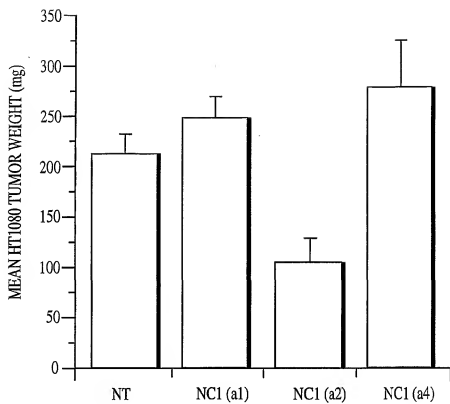


FIG. 12

13/30

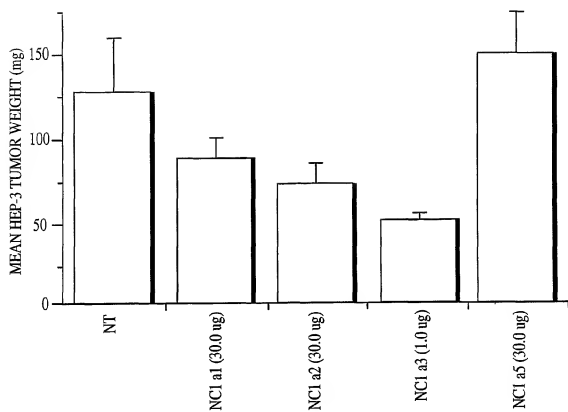


FIG. 13

14/30

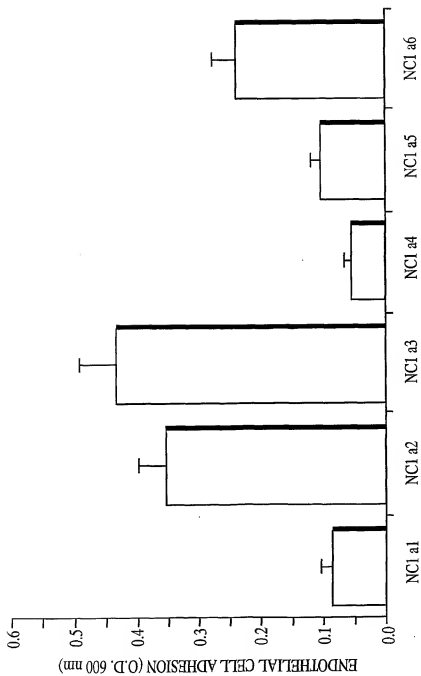


FIG. 14

15/30

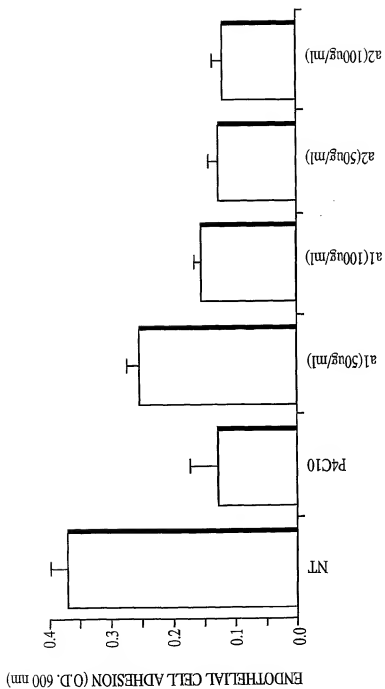


FIG. 15

16/30

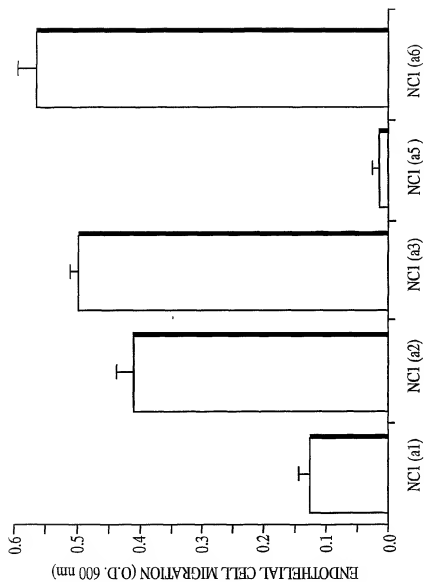


FIG. 16

A. $\alpha 1$ (IV) NC1

17/30

```

900      910      920      930      940      950
|         |         |         |         |
CTGCCGCGCTGCCTGCCTGCCACTGAGGGTTCCACGACCATGAGGCGCTGGATCTTCTTT
                M R A W I F F

960      970      980      990      1000     1010
|         |         |         |         |
CTCCTTTTCCTGGCCGGGAGGGCTCTGGCAGCCCCACTAGCGACTACAAGGACGACGAT
L L C L A G R A L A A P L A D Y K D D D

1020     1030     1040     1050     1060     1070
|         |         |         |         |
GACAAGCTAGCATCTGTTGATCACGGCTTCCTTGTGACCAGGCATAGTCAAAACAATAGAT
D K L A S V D H G F L V T R H S Q T I D

1080     1090     1100     1110     1120     1130
|         |         |         |         |
GACCCACAGTGTCTCTTCTGGGACCAAATTCCTTACCACGGGTACTCTTTGCTCTACGTG
D P Q C P S G T K I L Y H G Y S L L Y V

1140     1150     1160     1170     1180     1190
|         |         |         |         |
CAAGGCAATGAACGGGCCCATGGCCAGGACTTGGGCACGGCCGGCAGCTGCCTGCGCAAG
Q G N E R A H G Q D L G T A G S C L R K

1200     1210     1220     1230     1240     1250
|         |         |         |         |
TTCAGCACAAATGCCCTTCCTGTTCTGCAATATTAAACAAGTGTGCAACTTTGCATCACGA
F S T M P F L F C N I N N V C N F A S R

1260     1270     1280     1290     1300     1310
|         |         |         |         |
AATGACTACTCGTACTGGCTGTCCACCCCTGAGCCCATGCCCATGTCAATGGCACCCATC
N D Y S Y W L S T P E P M P M S M A P I

1320     1330     1340     1350     1360     1370
|         |         |         |         |
ACGGGGGAAACATAAGACCATTTATTAGTAGGTGTGCTGTGTGTGAGGCGCCTGCCATG
T G E N I R P F I S R C A V C E A P A M

1380     1390     1400     1410     1420     1430
|         |         |         |         |
GTGATGGCCGTGCACAGCCAGACCATTCAGATCCCACCGTGCCCAAGCGGTGGTCTCTG
V M A V H S Q T I Q I P P C P S G W S S

1440     1450     1460     1470     1480     1490
|         |         |         |         |
CTGTGGATCGGCTACTCTTTTGTGATGCACACCAGCGCTGGTGCAGAAGGCTCTGCCCAA
L W I G Y S F V M H T S A G A E G S G Q

1500     1510     1520     1530     1540     1550
|         |         |         |         |
GCCCTGGCGTCCCCCGGCTCTGCTGGAGAGTTTAGAAGTGCGCCATTTCATCGAGTGT

```

FIG. 17a

18/30

A L A S P G S C L E E F R S A P F I E C									
1560	1570	1580	1590	1600	1610				
CACGGCCGTGGGACCTGCAATTACTACGCAACGCTTACAGCTTTTGGCTCGCCACCATA									
H G R G T C N Y Y A N A Y S F W L A T I									
1620	1630	1640	1650	1660	1670				
GAGAGGAGCGAGATGTTCAAGAAGCCTACGCCGTCACCTTGAAGGCAGGGGAGCTGCGC									
E R S E M F K K P T P S T L K A G E L R									
1680	1690	1700	1710	1720	1730				
ACGCACGTCAGCCGCTGCCAAGTCTGTATGAGAAGAACATAATGAAGCCTGACTCAGCTA									
T H V S R C Q V C M R R T - -									
1740	1750	1760	1770	1780	1790				
CCGCGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTG									

FIG. 17a

19/30

B. $\alpha 2$ (IV) NC1

```

-----
900      910      920      930      940      950
CTGCCGCTGCCTGCCTGCCACTGAGGTTCCACGACCATGAGGCGCTGGATCTTCTTT
                                M R A W I F F

960      970      980      990      1000     1010
CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT
L L C L A G R A L A A P L A D Y K D D D

1020     1030     1040     1050     1060     1070
GACAAGCTAGCCGTCAGCATCGCTACCTCCTGGTGAAGCACAGCCAGACGGACCAGGAG
D K L A V S I G Y L L V K H S Q T D Q E

1080     1090     1100     1110     1120     1130
CCCATGTGCCCGTGGGCATGAACAACTCTGGAGTGGATACAGCCTGCTGTACTTTCGAG
P M C P V G M N K L W S G Y S L L Y F E

1140     1150     1160     1170     1180     1190
GGCCAGGAGAAGGCGCAACCCAGGACCTGGGGCTGGCGGGCTCCTGCCTGGCGCGTTTC
G Q E K A H N Q D L G L A G S C L A R F

1200     1210     1220     1230     1240     1250
AGCACCATGCCCTTCTCTACTGTCAACCCCTGGTGATGTCTGTACTATGCCAGCCGGAAC
S T M P F L Y C N P G D V C Y Y A S R N

1260     1270     1280     1290     1300     1310
GACAAGTCCTACTGGCTCTCTACCACTGCGCGCTGCCCATGATGCCCGTGCGCCAGGAC
D K S Y W L S T T A P L P M M P V A E D

1320     1330     1340     1350     1360     1370
GAGATCAAGCCCTACATCAGCCGCTGTCTGTGTGTGAGGCCCGGCCATGCCCATCGCG
E I K P Y I S R C S V C E A P A I A I A

1380     1390     1400     1410     1420     1430
GTCCACAGTCAGGATGTCTCCATCCCACTGCCAGCTGGGTGGCGGAGTTTGTGGATC
V H S Q D V S I P H C P A G W R S L W I

1440     1450     1460     1470     1480     1490
GGATATTCCTTCCTCATGCACGCGCGCGGAGACGAAGGCGGTGGCCAACTACTGTGT
G Y S F L M H T A A G D E G G G Q S L V

1500     1510     1520     1530     1540     1550

```

FIG. 17b

20/30

TCACCGGGCAGCTGTCTAGAGGACTTCGCGGCCACACCATTCATCGAATGCAATGGAGGC					
S P G S C L E D F R A T P F I E C N G G					
1560	1570	1580	1590	1600	1610
CGCGGCACCTGCGCACTACTACGCCAAACAAGTACAGCTTCTGGCTGACCACCATTCCTCCGAG					
R G T C H Y Y A N K Y S F W L T T I P E					
1620	1630	1640	1650	1660	1670
CAGAGCTTCCAGGGCTCGCCCTCCGCGGACACGCTCAAGGCCGGCTCATCCGCGACACAC					
Q S F Q G S P S A D T L K A G L I R T H					
1680	1690	1700	1710	1720	1730
ATCAGCCGCTGCCAGGTGTGCATGAAGAACCTGTGAGCCCGCGCGTGCCAGGGCCCTATT					
I S R C Q V C M K N L -					
1740	1750	1760	1770	1780	1790
CTATAGTGTCACTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGC					

FIG. 17b

21/30

C. $\alpha 3$ (IV)NC1

```

-----
900      910      920      930      940      950
CTGCCGCTGCCTGCCTGCCACTGAGGGTCCCGACACCATGAGGGCCTGGATCTTCTTT
                                     M R A W I F F

960      970      980      990      1000     1010
CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCGCTAGCCGACTACAAGGACGACGAT
L L C L A G R A L A A P L A D Y K D D D

1020     1030     1040     1050     1060     1070
GACAAACGTGGAGACAGTGGATCACCTGCCAACCTGGACAACGAGAGGCTTTGTCTTCACC
D K R G D S G S P A T W T T R G F V F T

1080     1090     1100     1110     1120     1130
CGACACAGTCAAACCCACAGCAATTCTCTCATGTCCAGAGGGGACAGTGCCACTCTCAGT
R H S Q T T A I P S C P E G T V P L Y S

1140     1150     1160     1170     1180     1190
GGGTTTCTTTCTTTTGTGTACAAGGAAATCAACGAGCCACGGACAAGACCTTGGAACT
G F S F L F V Q G N Q R A H G Q D L G T

1200     1210     1220     1230     1240     1250
CTTGGCAGCTGCCCTGCAGCGATTACACCAATGCCATCTTATTCTGCAATGTCAATGAT
L G S C L Q R F T T M P F L F C N V N D

1260     1270     1280     1290     1300     1310
GTATGTAATTTGCATCTCGAAATGATTATTCATACTGGCTGTCAACACCGCTCTGATG
V C N F A S R N D Y S Y W L S T P A L M

1320     1330     1340     1350     1360     1370
CCAATGAACATGGCTCCCATTA CTGGCAGGCCCTTGAGCCTTATATAAGCAGATGCACT
P M N M A P I T G R A L E P Y I S R C T

1380     1390     1400     1410     1420     1430
TTTGTGAAGGTCTCGCATCGCCATAGCCGTTACAGCCAAACCACTGACATCTCTCCA
V C E G P A I A I A V H S Q T D I P P

1440     1450     1460     1470     1480     1490
TGTCCTCAGCGCTGGATTCTCTCTGGAAGGATTTTCATTCATCATGTTCACAAGTGCA
C P H G W I S L W K G F S F I M F T S A

1500     1510     1520     1530     1540     1550

```

FIG. 17c

22/30

```
|         |         |         |         |         |
GGTTCTGAGGGCGCCGGGCAGCACTGGCCTCCCCGGCTCCTGCC'TGGAAGAATTCGA
G S E G A G Q A L A S P G S C L E E F R

1560      1570      1580      1590      1600      1610
GCCAGCCCATTTCTAGAATGTCATGGAAGAGGAACGTGCAACTACTATTCAAATTCCTAC
A S P F L E C H G R G T C N Y Y S N S Y

1620      1630      1640      1650      1660      1670
AGTTTCTGGCTGGCTTCATTAAACCCAGAAAGAAATGTTTCAGAAAGCCTATTCATCAACT
S F W L A S L N P E R M F R K P I P S T

1680      1690      1700      1710      1720      1730
GTGAAAGCTGGGGAATTAGAAAAAATRATAAGTCGCTGTCAGGTGTGCATGAAGAAAAGA
V K A G E L E K I I S R C Q V C M K K R

1740      1750      1760      1770      1780      1790
CACTGAGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGCTGATCAGCCTCGAC

H -
```

FIG. 17c

23/30

D. $\alpha 4$ (IV) NC1

```

-----
900      910      920      930      940      950
CTGCCGCCTGCCTGCCACTGAGGGTCCCAGCACCATGAGGGCCTGGATCTCTTT
                               M R A W I F F

960      970      980      990      1000     1010
CTCCTTTGCTGGCCGGGAGGGCTCTGSCAGCCCGCTAGCCGACTACAGGACGACGAT
L L C L A G R A L A A P L A D Y K D D D

1020     1030     1040     1050     1060     1070
GACAAGCCTGGATACCTCGGTGGCTTCCTCTGGTTCTCCACAGTCAGACGGACAGGAG
D K P G Y L G G F L L V L H S Q T D Q E

1080     1090     1100     1110     1120     1130
CCCACCTGCCCCCTGGGCATGCCCAGGCTCTGGACTGGGTATAGTCTGTTATACCTGGAA
P T C P L G M P R L W T G Y S L L Y L E

1140     1150     1160     1170     1180     1190
GGCAGAGAGAAAGCTCACAATCAAGACCTTGGTCTGGCAGGGTCTTGCCCTCCCGTATTT
G Q E K A H N Q D L G L A G S C L P V F

1200     1210     1220     1230     1240     1250
AGCACGCTGCCCTTTGCCTACTGCAACATCCACCAGGTGTGCCACTATGCCCCAGAGAAAC
S T L P F A Y C N I H Q V C H Y A Q R N

1260     1270     1280     1290     1300     1310
GACAGATCCTACTGGCTGGCCAGCGCTGCGCCCTCCCATGATGCCACTCTCTGAAGAG
D R S Y W L A S A A P L P M M P L S E E

1320     1330     1340     1350     1360     1370
GCGATCCGCCCTATGTGACCGCTGTGCGGTATGCGAGGCCCGGCCAGGCGGTGGCG
A I R P Y V S R C A V C E A P A Q A V A

1380     1390     1400     1410     1420     1430
GTGCACAGCCAGGACCACTCCATCCCCCATGTCCGCGAGACCTGGAGGAGCCTCTGGATC
V H S Q D Q S I P P C P Q T W R S L W I

1440     1450     1460     1470     1480     1490
GGGTATTTCATTCTGTATGCACACAGGAGCTGGGGACCAAGGAGGAGGGCAGGCCCTTATG
G Y S F L M H T G A G D Q G G G Q A L M

1500     1510     1520     1530     1540     1550

```

FIG. 17d

T C A C C T T G G C A G C T G C C T G G A A G A T T T C A G A G C A G C A C C A T T C C T T G A A T G C C A G G G C C G G
 S P G S C L E D F R A A P F L E C Q G R
 1560 1570 1580 1590 1600 1610
 C A G G G A A C T T G C C A C T T T T T C G C A A T A A G T A T A G C T T C T G G C T C A C A C G G T G A A A G C A
 Q G T C H F F A N K Y S F W L T T V K A
 1620 1630 1640 1650 1660 1670
 G A C T T G C A G T T T T C T C T G C T C C A G C A C C A G A C A C C T T A A A A G A A A G C C A G G C C A A C G C
 D L Q F S S A P A P D T L K E S Q A Q R
 1680 1690 1700 1710 1720 1730
 C A G A A A T C A G C C G G T G C C A G G T C T G C G T G A A G T A T A G C T A G G G G C C C T A T T C T A T A G T G
 Q K I S R C Q V C V K Y S -
 1740 1750 1760 1770 1780 1790
 T C A C C T A A A T G C T A G A C T C G T G A T C A G C C T C G A C T G T G C C T T C T A G T T G C C A G C C A T C

SUBSTITUTE SHEET (RULE 26)

26/30

GGCTCAGGTCAAGCCCTAGCCTCCCTGGTTCCCTGCTGGAAGAGTTTCGTTTCAGCTCCC
G S G Q A L A S P G S C L E E F R S A P

1560 1570 1580 1590 1600 1610
TTCATCGAATGTCATGGGAGGGGTACCTGTAACTACTATGCCAACTCCTACAGCTTTTGG
F I E C H G R G T C N Y Y A N S Y S F W

1620 1630 1640 1650 1660 1670
CTGGCAACTGTAGATGTGTGACACATGTTTCAGTAAACCTCAGTCAGAAACGCTGAAAGCA
L A T V D V S D M F S K P Q S E T L K A

1680 1690 1700 1710 1720 1730
GGGAGACTTGAGGACACGAATTAGCCGATGTCAAGTGTGCATGAAGAGGACATAACGCGGC
G D L R T R I S R C Q V C M K R T

1740 1750 1760 1770 1780 1790
CGCTCGAGCATGCATCTAGAGGGCCCTATTCTATAGTGTACCTAAATGCTAGAGCTCGC

FIG. 17e

28/30

GTCTCACCTGGCTCCTGCCTAGAGGACTTTGGGGCACTCCTTTCATCGAATGCAGTGGT
V S P G S C L E D F R A T P F I E C S G

1560 1570 1580 1590 1600 1610
GCCCGAGGCACCTGCCACTACTTTGCAACAAGTACAGTTTCTGGTTGACCACAGTGGAG
A R G T C H Y F A N K Y S F W L T T V E

1620 1630 1640 1650 1660 1670
GAGAGGCAGCAGTTTGGGGAGTTGCCTGTGTCTGAAACGCTGAAAGCTGGGCAGCTCCAC
E R Q Q F G E L P V S E T L K A G Q L H

1680 1690 1700 1710 1720 1730
ACTCGAGTCAGTCGCTGCCAGGTGTGTATGAAAAGCCTGTAGGGTGGCACCTGCCACGGG
T R V S R C Q V C M K S L -

1740 1750 1760 1770 1780 1790
CCCTATTCTATAGTGTACCTAAATGCTAGAGCTGCTGATCAGCCTCGACTGTGCCTTC

FIG. 17f

29/30

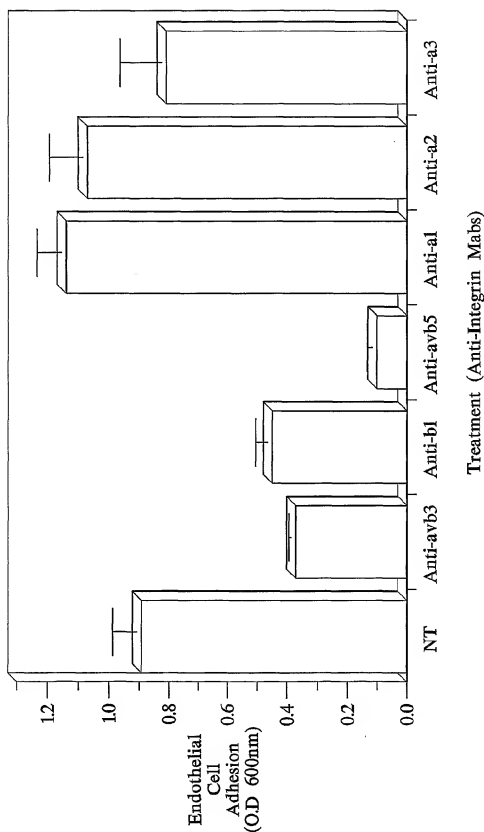
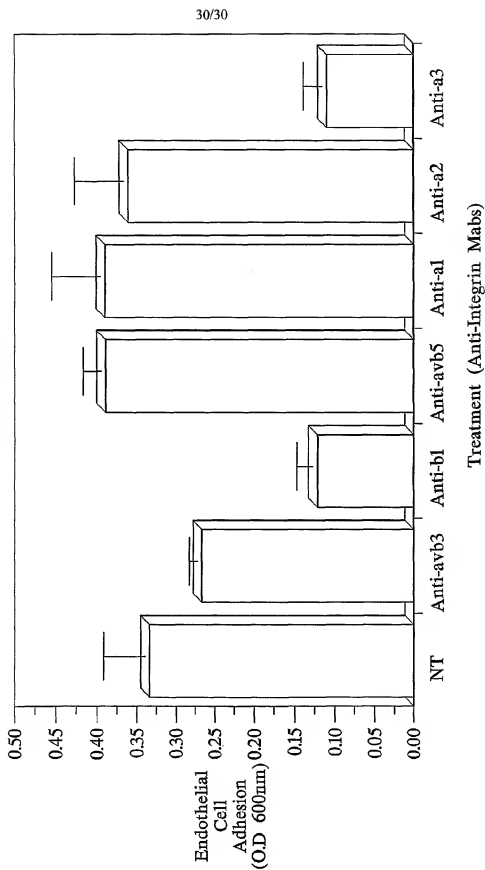
FIG. 18

FIG. 19

SEQUENCE LISTING

<110> Biostratum, Inc. et al.

<120> Methods to Inhibit Angiogenesis and Tumor Growth

<130> 94525K2

<140> To be assigned

<141> Herewith

<160> 12

<170> PatentIn Ver. 2.0

<210> 1

<211> 900

<212> DNA

<213> Human

<220>

<221> CDS

<222> (40)..(819)

<400> 1

```

ctgccgctg cctgcctgcc actgaggggt cccagcacc atg agg gcc tgg atc   54
                               Met Arg Ala Trp Ile
                               1           5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc cca cta gcc   102
Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
                10                15                20

gac tac aag gac gac gat gac aag cta gca tct gtt gat cac ggc ttc   150
Asp Tyr Lys Asp Asp Asp Lys Leu Ala Ser Val Asp His Gly Phe
                25                30                35

ctt gtg acc agg cat agt caa aca ata gat gac cca cag tgt cct tct   198
Leu Val Thr Arg His Ser Gln Thr Ile Asp Asp Pro Gln Cys Pro Ser
                40                45                50

ggg acc aaa att ctt tac cac ggg tac tct ttg ctc tac gtg caa ggc   246
Gly Thr Lys Ile Leu Tyr His Gly Tyr Ser Leu Leu Tyr Val Gln Gly
                55                60                65

aat gaa cgg gcc cat ggc cag gac ttg ggc acg gcc ggc agc tgc ctg   294
Asn Glu Arg Ala His Gly Gln Asp Leu Gly Thr Ala Gly Ser Cys Leu
                70                75                80                85

cgc aag ttc agc aca atg ccc ttc ctg ttc tgc aat att aac aac gtg   342
Arg Lys Phe Ser Thr Met Pro Phe Leu Phe Cys Asn Ile Asn Asn Val
                90                95                100

tgc aac ttt gca tca cga aat gac tac tgc tac tgg ctg tcc acc cct   390
Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp Leu Ser Thr Pro
                105                110                115

```

gag ccc atg ccc atg tca atg gca ccc atc acg ggg gaa aac ata aga 438
 Glu Pro Met Pro Met Ser Met Ala Pro Ile Thr Gly Glu Asn Ile Arg
 120 125 130

cca ttt att agt agg tgt gct gtg tgt gag gcg cct gcc atg gtg atg 486
 Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala Pro Ala Met Val Met
 135 140 145

gcc gtg cac agc cag acc att cag atc cca cgg tgc ccc agc ggg tgg 534
 Ala Val His Ser Gln Thr Ile Gln Ile Pro Pro Cys Pro Ser Gly Trp
 150 155 160 165

tcc tcg ctg tgg atc ggc tac tct ttt gtg atg cac acc agc gct ggt 582
 Ser Ser Leu Trp Ile Gly Tyr Ser Phe Val Met His Thr Ser Ala Gly
 170 175 180

gca gaa ggc tct ggc caa gcc ctg gcg tcc ccc ggc tcc tgc ctg gag 630
 Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro Gly Ser Cys Leu Glu
 185 190 195

gag ttt aga agt gcg cca ttc atc gag tgt cac ggc cgt ggg acc tgc 678
 Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His Gly Arg Gly Thr Cys
 200 205 210

aat tac tac gca aac gct tac agc ttt tgg ctc gcc acc ata gag agg 726
 Asn Tyr Tyr Ala Asn Ala Tyr Ser Phe Trp Leu Ala Thr Ile Glu Arg
 215 220 225

agc gag atg ttc aag aag cct acg cgg tcc acc ttg aag gca ggg gag 774
 Ser Glu Met Phe Lys Lys Pro Thr Pro Ser Thr Leu Lys Ala Gly Glu
 230 235 240 245

ctg cgc acg cac gtc agc cgc tgc caa gtc tgt atg aga aga aca 819
 Leu Arg Thr His Val Ser Arg Cys Gln Val Cys Met Arg Arg Thr
 250 255 260

taatgaagcc tgactcagct accgcggggc ctattctata gtgtcaccta aatgctagag 879
 ctgcctgatc agcctcgact g 900

<210> 2
 <211> 260
 <212> PRT
 <213> Human

<400> 2
 Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
 1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Ser
 20 25 30

Val Asp His Gly Phe Leu Val Thr Arg His Ser Gln Thr Ile Asp Asp
 35 40 45

Pro Gln Cys Pro Ser Gly Thr Lys Ile Leu Tyr His Gly Tyr Ser Leu

Phe	Phe	Leu	Leu	Cys	Leu	Ala	Gly	Arg	Ala	Leu	Ala	Ala	Pro	Leu	Ala		
				10						15				20			
gac	tac	aag	gac	gac	gat	gac	aag	cta	gcc	gtc	agc	atc	ggc	tac	ctc	150	
Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Leu	Ala	Val	Ser	Ile	Gly	Tyr	Leu		
			25					30					35				
ctg	gtg	aag	cac	agc	cag	acg	gac	cag	gag	ccc	atg	tgc	ccg	gtg	ggc	198	
Leu	Val	Lys	His	Ser	Gln	Thr	Asp	Gln	Glu	Pro	Met	Cys	Pro	Val	Gly		
		40					45					50					
atg	aac	aaa	ctc	tgg	agt	gga	tac	agc	ctg	ctg	tac	ttc	gag	ggc	cag	246	
Met	Asn	Lys	Leu	Trp	Ser	Gly	Tyr	Ser	Leu	Leu	Tyr	Phe	Glu	Gly	Gln		
		55				60					65						
gag	aag	gcg	cac	aac	cag	gac	ctg	ggg	ctg	gcg	ggc	tcc	tgc	ctg	gcg	294	
Glu	Lys	Ala	His	Asn	Gln	Asp	Leu	Gly	Leu	Ala	Gly	Ser	Cys	Leu	Ala		
	70				75					80				85			
cgg	ttc	agc	acc	atg	ccc	ttc	ctg	tac	tgc	aac	cct	ggg	gat	gtc	tgc	342	
Arg	Phe	Ser	Thr	Met	Pro	Phe	Leu	Tyr	Cys	Asn	Pro	Gly	Asp	Val	Cys		
				90					95				100				
tac	tat	gcc	agc	cgg	aac	gac	aag	tcc	tac	tgg	ctc	tct	acc	act	gcg	390	
Tyr	Tyr	Ala	Ser	Arg	Asn	Asp	Lys	Ser	Tyr	Trp	Leu	Ser	Thr	Thr	Ala		
		105					110					115					
ccg	ctg	ccc	atg	atg	ccc	gtg	gcc	gag	gac	gag	atc	aag	ccc	tac	atc	438	
Pro	Leu	Pro	Met	Met	Pro	Val	Ala	Glu	Asp	Glu	Ile	Lys	Pro	Tyr	Ile		
		120					125					130					
agc	cgc	tgt	tct	gtg	tgt	gag	gcc	ccg	gcc	atc	gcc	atc	gcg	gtc	cac	486	
Ser	Arg	Cys	Ser	Val	Cys	Glu	Ala	Pro	Ala	Ile	Ala	Ile	Ala	Val	His		
		135				140					145						
agt	cag	gat	gtc	tcc	atc	cca	cac	tgc	cca	gct	ggg	tgg	cgg	agt	ttg	534	
Ser	Gln	Asp	Val	Ser	Ile	Pro	His	Cys	Pro	Ala	Gly	Trp	Arg	Ser	Leu		
	150				155				160					165			
tgg	atc	gga	tat	tcc	ttc	ctc	atg	cac	acg	gcg	gcg	gga	gac	gaa	ggc	582	
Trp	Ile	Gly	Tyr	Ser	Phe	Leu	Met	His	Thr	Ala	Ala	Gly	Asp	Glu	Gly		
			170						175				180				
ggg	ggc	caa	tca	ctg	gtg	tca	ccg	ggc	agc	tgt	cta	gag	gac	ttc	cgc	630	
Gly	Gly	Gln	Ser	Leu	Val	Ser	Pro	Gly	Ser	Cys	Leu	Glu	Asp	Phe	Arg		
		185					190					195					
gcc	aca	cca	ttc	atc	gaa	tgc	aat	gga	ggc	cgc	ggc	acc	tgc	cac	tac	678	
Ala	Thr	Pro	Phe	Ile	Glu	Cys	Asn	Gly	Gly	Arg	Gly	Thr	Cys	His	Tyr		
		200					205					210					
tac	gcc	aac	aag	tac	agc	ttc	tgg	ctg	acc	acc	att	ccc	gag	cag	agc	726	
Tyr	Ala	Asn	Lys	Tyr	Ser	Phe	Trp	Leu	Thr	Thr	Ile	Pro	Glu	Gln	Ser		
		215				220					225						
ttc	cag	ggc	tgc	ccc	tcc	gcc	gac	acg	ctc	aag	gcc	ggc	ctc	atc	cgc	774	
Phe	Gln	Gly	Ser	Pro	Ser	Ala	Asp	Thr	Leu	Lys	Ala	Gly	Leu	Ile	Arg		

5

230	235	240	245	
aca cac atc agc cgc tgc cag gtg tgc atg aag aac ctg tgagccggcg				823
Thr His Ile Ser Arg Cys Gln Val Cys Met Lys Asn Leu				
	250	255		
cgtagccaggg ccctattcta tagtgtcacc taaatgctag agctcgctga tcagcctcga				883
ctctgccttc tagttgc				900

<210>	4
<211>	258
<212>	PRT
<213>	Human

400> 4	Met	Arg	Ala	Trp	Ile	Phe	Phe	Leu	Leu	Cys	Leu	Ala	Gly	Arg	Ala	Leu
1					5					10					15	
Ala	Ala	Pro	Leu	Ala	Asp	Tyr	Lys	Asp	Asp	Asp	Asp	Lys	Leu	Ala	Val	
			20					25					30			
Ser	Ile	Gly	Tyr	Leu	Leu	Val	Lys	His	Ser	Gln	Thr	Asp	Gln	Glu	Pro	
		35					40					45				
Met	Cys	Pro	Val	Gly	Met	Asn	Lys	Leu	Trp	Ser	Gly	Tyr	Ser	Leu	Leu	
	50					55					60					
Tyr	Phe	Glu	Gly	Gln	Glu	Lys	Ala	His	Asn	Gln	Asp	Leu	Gly	Leu	Ala	
65					70					75					80	
Gly	Ser	Cys	Leu	Ala	Arg	Phe	Ser	Thr	Met	Pro	Phe	Leu	Tyr	Cys	Asn	
			85						90					95		
Pro	Gly	Asp	Val	Cys	Tyr	Tyr	Ala	Ser	Arg	Asn	Asp	Lys	Ser	Tyr	Trp	
			100					105					110			
Leu	Ser	Thr	Thr	Ala	Pro	Leu	Pro	Met	Met	Pro	Val	Ala	Glu	Asp	Glu	
		115				120						125				
Ile	Lys	Pro	Tyr	Ile	Ser	Arg	Cys	Ser	Val	Cys	Glu	Ala	Pro	Ala	Ile	
	130					135					140					
Ala	Ile	Ala	Val	His	Ser	Gln	Asp	Val	Ser	Ile	Pro	His	Cys	Pro	Ala	
145					150					155					160	
Gly	Trp	Arg	Ser	Leu	Trp	Ile	Gly	Tyr	Ser	Phe	Leu	Met	His	Thr	Ala	
			165						170					175		
Ala	Gly	Asp	Glu	Gly	Gly	Gly	Gln	Ser	Leu	Val	Ser	Pro	Gly	Ser	Cys	
			180					185					190			
Leu	Glu	Asp	Phe	Arg	Ala	Thr	Pro	Phe	Ile	Glu	Cys	Asn	Gly	Gly	Arg	
	195					200						205				
Gly	Thr	Cys	His	Tyr	Tyr	Ala	Asn	Lys	Tyr	Ser	Phe	Trp	Leu	Thr	Thr	

210 215 220

Ile Pro Glu Gln Ser Phe Gln Gly Ser Pro Ser Ala Asp Thr Leu Lys
 225 230 235 240

Ala Gly Leu Ile Arg Thr His Ile Ser Arg Cys Gln Val Cys Met Lys
 245 250 255

Asn Leu

<210> 5
 <211> 900
 <212> DNA
 <213> Human

<220>
 <221> CDS
 <222> (40)..(843)

<400> 5
 ctgccgcctg cctgectgcc actgagggtt cccagcacc atg agg gcc tgg atc 54
 Met Arg Ala Trp Ile
 1 5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc cgg cta gcc 102
 Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
 10 15 20

gac tac aag gac gac gat gac aaa cgt gga gac agt gga tca cct gca 150
 Asp Tyr Lys Asp Asp Asp Lys Arg Gly Asp Ser Gly Ser Pro Ala
 25 30 35

acc tgg aca acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca 198
 Thr Trp Thr Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr
 40 45 50

gca att cct tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt 246
 Ala Ile Pro Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe
 55 60 65

tct ttt ctt ttt gta caa gga aat caa cga gcc cac gga caa gac ctt 294
 Ser Phe Leu Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu
 70 75 80 85

gga act ctt ggc agc tgc ctg cag cga ttt acc aca atg cca ttc tta 342
 Gly Thr Leu Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu
 90 95 100

ttc tgc aat gtc aat gat gta tgt aat ttt gca tct cga aat gat tat 390
 Phe Cys Asn Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr
 105 110 115

tca tac tgg ctg tca aca cca gct ctg atg cca atg aac atg gct ccc 438
 Ser Tyr Trp Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro
 120 125 130

att act ggc aga gcc ctt gag cct tat ata agc aga tgc act gtt tgt 486
 ile Thr Gly Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys
 135 140 145
 gaa ggt cct gcg atc gcc ata gcc gtt cac agc caa acc act gac att 534
 Glu Gly Pro Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile
 150 155 160 165
 cct cca tgt cct cac ggc tgg att tct ctc tgg aaa gga ttt tca ttc 582
 Pro Pro Cys Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe
 170 175 180
 atc atg ttc aca agt gca ggt tct gag ggc gcc ggg caa gca ctg gcc 630
 ile Met Phe Thr Ser Ala Gly Ser Glu Gly Ala Gly Gln Ala Leu Ala
 185 190 195
 tcc ccc ggc tcc tgc ctg gaa gaa ttc cga gcc agc cca ttt cta gaa 678
 Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ala Ser Pro Phe Leu Glu
 200 205 210
 tgt cat gga aga gga acg tgc aac tac tat tca aat tcc tac agt ttc 726
 Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ser Asn Ser Tyr Ser Phe
 215 220 225
 tgg ctg gct tca tta aac cca gaa aga atg ttc aga aag cct att cca 774
 Trp Leu Ala Ser Leu Asn Pro Glu Arg Met Phe Arg Lys Pro Ile Pro
 230 235 240 245
 tca act gtg aaa gct ggg gaa tta gaa aaa ata ata agt cgc tgt cag 822
 Ser Thr Val Lys Ala Gly Glu Leu Glu Lys Ile Ile Ser Arg Cys Gln
 250 255 260
 gtg tgc atg aag aaa aga cac tgagggccct attctatagt gtcacataaa 873
 Val Cys Met Lys Lys Arg His
 265
 tgctagagct cgctgacgac cctcgac 900

 <210> 6
 <211> 268
 <212> PRT
 <213> Human

 <400> 6
 Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
 1 5 10 15
 Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Arg Gly Asp
 20 25 30
 Ser Gly Ser Pro Ala Thr Trp Thr Thr Arg Gly Phe Val Phe Thr Arg
 35 40 45
 His Ser Gln Thr Thr Ala Ile Pro Ser Cys Pro Glu Gly Thr Val Pro
 50 55 60

Leu Tyr Ser Gly Phe Ser Phe Leu Phe Val Gln Gly Asn Gln Arg Ala
 65 70 75 80
 His Gly Gln Asp Leu Gly Thr Leu Gly Ser Cys Leu Gln Arg Phe Thr
 85 90 95
 Thr Met Pro Phe Leu Phe Cys Asn Val Asn Asp Val Cys Asn Phe Ala
 100 105 110
 Ser Arg Asn Asp Tyr Ser Tyr Trp Leu Ser Thr Pro Ala Leu Met Pro
 115 120 125
 Met Asn Met Ala Pro Ile Thr Gly Arg Ala Leu Glu Pro Tyr Ile Ser
 130 135 140
 Arg Cys Thr Val Cys Glu Gly Pro Ala Ile Ala Ile Ala Val His Ser
 145 150 155 160
 Gln Thr Thr Asp Ile Pro Pro Cys Pro His Gly Trp Ile Ser Leu Trp
 165 170 175
 Lys Gly Phe Ser Phe Ile Met Phe Thr Ser Ala Gly Ser Glu Gly Ala
 180 185 190
 Gly Gln Ala Leu Ala Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ala
 195 200 205
 Ser Pro Phe Leu Glu Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ser
 210 215 220
 Asn Ser Tyr Ser Phe Trp Leu Ala Ser Leu Asn Pro Glu Arg Met Phe
 225 230 235 240
 Arg Lys Pro Ile Pro Ser Thr Val Lys Ala Gly Glu Leu Glu Lys Ile
 245 250 255
 Ile Ser Arg Cys Gln Val Cys Met Lys Lys Arg His
 260 265

<210> 7
 <211> 900
 <212> DNA
 <213> Human

<220>
 <221> CDS
 <222> (40)..(819)

<400> 7
 ctgcgcgctg cctgcctgcc actgaggggtt cccagcacc atg agg gcc tgg atc 54
 Met Arg Ala Trp Ile
 1 5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc ceg cta gcc 102
 Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala

															9																	
										10					15					20												
gac	tac	aag	gac	gac	gat	gac	aag	cct	gga	tac	ctc	ggt	ggc	ttc	ctc	150																
Asp	Tyr	Lys	Asp	Asp	Asp	Lys	Pro	Gly	Tyr	Leu	Gly	Gly	Phe	Leu																		
																25	30		35													
ctg	gtt	ctc	cac	agt	cag	acg	gac	cag	gag	ccc	acc	tgc	ccc	ctg	ggc	198																
Leu	Val	Leu	His	Ser	Gln	Thr	Asp	Gln	Glu	Pro	Thr	Cys	Pro	Leu	Gly																	
																40	45		50													
atg	ccc	agg	ctc	tgg	act	ggg	tat	agt	ctg	tta	tac	ctg	gaa	ggg	caa	246																
Met	Pro	Arg	Leu	Trp	Thr	Gly	Tyr	Ser	Leu	Leu	Tyr	Leu	Glu	Gly	Gln																	
																55	60		65													
gag	aaa	gct	cac	aat	caa	gac	ctt	ggt	ctg	gca	ggg	tct	tgc	ctt	ccc	294																
Glu	Lys	Ala	His	Asn	Gln	Asp	Leu	Gly	Leu	Ala	Gly	Ser	Cys	Leu	Pro																	
																70	75		80		85											
gta	ttt	agc	acg	ctg	ccc	ttt	gcc	tac	tgc	aac	atc	cac	cag	gtg	tgc	342																
Val	Phe	Ser	Thr	Leu	Pro	Phe	Ala	Tyr	Cys	Asn	Ile	His	Gln	Val	Cys																	
																90	95		100													
cac	tat	gcc	cag	aga	aac	gac	aga	tcc	tac	tgg	ctg	gcc	agc	gct	gcg	390																
His	Tyr	Ala	Gln	Arg	Asn	Asp	Ser	Tyr	Trp	Leu	Ala	Ser	Ala	Ala																		
																105	110		115													
ccc	ctc	ccc	atg	atg	cca	ctc	tct	gaa	gag	gcg	atc	cgc	ccc	tat	gtc	438																
Pro	Leu	Pro	Met	Met	Pro	Leu	Ser	Glu	Glu	Ala	Ile	Arg	Pro	Tyr	Val																	
																120	125		130													
agc	cgc	tgt	gcg	gta	tgc	gag	gcc	ccg	gcc	cag	gcg	gtg	gcg	gtg	cac	486																
Ser	Arg	Cys	Ala	Val	Cys	Glu	Ala	Pro	Ala	Gln	Ala	Val	Ala	Val	His																	
																135	140		145													
agc	cag	gac	cag	tcc	atc	ccc	cca	tgt	ccg	cag	acc	tgg	agg	agc	ctc	534																
Ser	Gln	Asp	Gln	Ser	Ile	Pro	Pro	Cys	Pro	Gln	Thr	Trp	Arg	Ser	Leu																	
																150	155		160		165											
tgg	atc	ggg	tat	tca	ttc	ctg	atg	cac	aca	gga	gct	ggg	gac	caa	gga	582																
Trp	Ile	Gly	Tyr	Ser	Phe	Leu	Met	His	Thr	Gly	Ala	Gly	Asp	Gln	Gly																	
																170	175		180													
gga	ggg	cag	gcc	ctt	atg	tca	cct	ggc	agc	tgc	ctg	gaa	gat	ttc	aga	630																
Gly	Gly	Gln	Ala	Leu	Met	Ser	Pro	Gly	Ser	Cys	Leu	Glu	Asp	Phe	Arg																	
																185	190		195													
gca	gca	cca	ttc	ctt	gaa	tgc	cag	ggc	cgg	cag	gga	act	tgc	cac	ttt	678																
Ala	Ala	Pro	Phe	Leu	Glu	Cys	Gln	Gly	Arg	Gln	Gly	Thr	Cys	His	Phe																	
																200	205		210													
ttc	gca	aat	aag	tat	agc	ttc	tgg	ctc	aca	acg	gtg	aaa	gca	gac	ttg	726																
Phe	Ala	Asn	Lys	Tyr	Ser	Phe	Trp	Leu	Thr	Thr	Val	Lys	Ala	Asp	Leu																	
																215	220		225													
cag	ttt	tcc	tct	gct	cca	gca	cca	gac	acc	tta	aaa	gaa	agc	cag	gcc	774																
Gln	Phe	Ser	Ser	Ala	Pro	Ala	Pro	Asp	Thr	Leu	Lys	Glu	Ser	Gln	Ala																	
																230	235		240		245											

caa cgc cag aaa atc agc cgg tgc cag gtc tgc gtc aag tat agc 819
 Gln Arg Gln Lys Ile Ser Arg Cys Gln Val Cys Val Lys Tyr Ser
 250 255 260

taggggacct attctatagt gtcacctaaa tgctagagct cgctgatcag cctcgactgt 879

gccttctagt tgccagccat c 900

<210> 8
 <211> 260
 <212> PRT
 <213> Human

<400> 8
 Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
 1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Pro Gly Tyr
 20 25 30

Leu Gly Gly Phe Leu Leu Val Leu His Ser Gln Thr Asp Gln Glu Pro
 35 40 45

Thr Cys Pro Leu Gly Met Pro Arg Leu Trp Thr Gly Tyr Ser Leu Leu
 50 55 60

Tyr Leu Glu Gly Gln Glu Lys Ala His Asn Gln Asp Leu Gly Leu Ala
 65 70 75 80

Gly Ser Cys Leu Pro Val Phe Ser Thr Leu Pro Phe Ala Tyr Cys Asn
 85 90 95

Ile His Gln Val Cys His Tyr Ala Gln Arg Asn Asp Arg Ser Tyr Trp
 100 105 110

Leu Ala Ser Ala Ala Pro Leu Pro Met Met Pro Leu Ser Glu Glu Ala
 115 120 125

Ile Arg Pro Tyr Val Ser Arg Cys Ala Val Cys Glu Ala Pro Ala Gln
 130 135 140

Ala Val Ala Val His Ser Gln Asp Gln Ser Ile Pro Pro Cys Pro Gln
 145 150 155 160

Thr Trp Arg Ser Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr Gly
 165 170 175

Ala Gly Asp Gln Gly Gly Gly Gln Ala Leu Met Ser Pro Gly Ser Cys
 180 185 190

Leu Glu Asp Phe Arg Ala Ala Pro Phe Leu Glu Cys Gln Gly Arg Gln
 195 200 205

Gly Thr Cys His Phe Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr
 210 215 220

Val Lys Ala Asp Leu Gln Phe Ser Ser Ala Pro Ala Pro Asp Thr Leu
 225 230 235 240

Lys Glu Ser Gln Ala Gln Arg Gln Lys Ile Ser Arg Cys Gln Val Cys
 245 250 255

Val Lys Tyr Ser
 260

<210> 9

<211> 900

<212> DNA

<213> Human

<220>

<221> CDS

<222> (40)..(831)

<400> 9

ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc 54
 Met Arg Ala Trp Ile
 1 5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc ccg cta gct 102
 Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
 10 15 20

gac tac aag gac gac gat gac aaa ggt ccc cct gga acc tcc tct gtt 150
 Asp Tyr Lys Asp Asp Asp Lys Gly Pro Pro Gly Thr Ser Ser Val
 25 30 35

gca cat gga ttt ctt att aca cgc cac agc cag aca acg gat gca cca 198
 Ala His Gly Phe Leu Ile Thr Arg His Ser Gln Thr Thr Asp Ala Pro
 40 45 50

caa tgc cca cag gga aca ctt cag gtc tat gaa ggc ttt tct ctc ctg 246
 Gln Cys Pro Gln Gly Thr Leu Gln Val Tyr Glu Gly Phe Ser Leu Leu
 55 60 65

tat gta caa gga aat aaa aga gcc cac ggt caa gac ttg ggg acg gct 294
 Tyr Val Gln Gly Asn Lys Arg Ala His Gly Gln Asp Leu Gly Thr Ala
 70 75 80 85

ggc agc tgc ctt cgt cgc ttt agt acc atg cct ttc atg ttc tgc aac 342
 Gly Ser Cys Leu Arg Arg Phe Ser Thr Met Pro Phe Met Phe Cys Asn
 90 95 100

atc aat aat gtt tgc aac ttt gct tca aga aat gac tat tct tac tgg 390
 Ile Asn Asn Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
 105 110 115

ctc tct acc cca gag ccc atg cca atg agc atg caa ccc cta aag ggc 438
 Leu Ser Thr Pro Glu Pro Met Pro Met Ser Met Gln Pro Leu Lys Gly
 120 125 130

cag agc atc cag cca ttc att agt cga tgt gca gta tgt gaa gct cca 486
 Gln Ser Ile Gln Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala Pro
 135 140 145

gct gtg gtg atc gca gtt cac agt cag acg atc cag att ccc cat tgt 534
 Ala Val Val Ile Ala Val His Ser Gln Thr Ile Gln Ile Pro His Cys
 150 155 160 165

cct cag gga tgg gat tct ctg tgg att ggt tat tcc ttc atg atg cat 582
 Pro Gln Gly Trp Asp Ser Leu Trp Ile Gly Tyr Ser Phe Met Met His
 170 175 180

aca agt gca ggg gca gaa ggc tca ggt caa gcc cta gcc tcc cct ggt 630
 Thr Ser Ala Gly Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro Gly
 185 190 195

tcc tgc ttg gaa gag ttt cgt tca gct ccc ttc atc gaa tgt cat ggg 678
 Ser Cys Leu Glu Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His Gly
 200 205 210

agg ggt acc tgt aac tac tat gcc aac tcc tac agc ttt tgg ctg gca 726
 Arg Gly Thr Cys Asn Tyr Tyr Ala Asn Ser Tyr Ser Phe Trp Leu Ala
 215 220 225

act gta gat gtg tca gac atg ttc agt aaa cct cag tca gaa acg ctg 774
 Thr Val Asp Val Ser Asp Met Phe Ser Lys Pro Gln Ser Glu Thr Leu
 230 235 240 245

aaa gca gga gac ttg agg aca cga att agc cga tgt caa gtg tgc atg 822
 Lys Ala Gly Asp Leu Arg Thr Arg Ile Ser Arg Cys Gln Val Cys Met
 250 255 260

aag agg aca taacgaggcc gctcagcat gcacatagag ggccctattc 871
 Lys Arg Thr

tatagtgtca cctaaatgct agagctcgc 900

<210> 10
 <211> 264
 <212> PRT
 <213> Human

<400> 10
 Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
 1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Gly Pro Pro
 20 25 30

Gly Thr Ser Ser Val Ala His Gly Phe Leu Ile Thr Arg His Ser Gln
 35 40 45

Thr Thr Asp Ala Pro Gln Cys Pro Gln Gly Thr Leu Gln Val Tyr Glu
 50 55 60

Gly Phe Ser Leu Leu Tyr Val Gln Gly Asn Lys Arg Ala His Gly Gln

	65	70	75	80
Asp Leu Gly Thr Ala Gly Ser Cys Leu Arg Arg Phe Ser Thr Met Pro 85 90 95				
Phe Met Phe Cys Asn Ile Asn Asn Val Cys Asn Phe Ala Ser Arg Asn 100 105 110				
Asp Tyr Ser Tyr Trp Leu Ser Thr Pro Glu Pro Met Pro Met Ser Met 115 120 125				
Gln Pro Leu Lys Gly Gln Ser Ile Gln Pro Phe Ile Ser Arg Cys Ala 130 135 140				
Val Cys Glu Ala Pro Ala Val Val Ile Ala Val His Ser Gln Thr Ile 145 150 155 160				
Gln Ile Pro His Cys Pro Gln Gly Trp Asp Ser Leu Trp Ile Gly Tyr 165 170 175				
Ser Phe Met Met His Thr Ser Ala Gly Ala Glu Gly Ser Gly Gln Ala 180 185 190				
Leu Ala Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ser Ala Pro Phe 195 200 205				
Ile Glu Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ala Asn Ser Tyr 210 215 220				
Ser Phe Trp Leu Ala Thr Val Asp Val Ser Asp Met Phe Ser Lys Pro 225 230 235 240				
Gln Ser Glu Thr Leu Lys Ala Gly Asp Leu Arg Thr Arg Ile Ser Arg 245 250 255				
Cys Gln Val Cys Met Lys Arg Thr 260				
<210> 11				
<211> 900				
<212> DNA				
<213> Human				
<220>				
<221> CDS				
<222> (40)..(819)				
<400> 11				
cctgcgacctg cctgcctgcc actgagggtt ccagcacc atg agg gcc tgg atc Met Arg Ala Trp Ile 54 1 5				
ttc ttt ctg ctt tgc ctg gcc ggg agg gct ctg gca gcc cca cta gcc Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala 102 10 15 20				

gac tac aag gac gac gat gac aag cta gcg agc atg aga gtg ggc tac	150
Asp Tyr Lys Asp Asp Asp Lys Leu Ala Ser Met Arg Val Gly Tyr	
25 30 35	
acg ttg gta aag cac agc cag tcg gaa cag gtg ccc ccg tgt ccc atc	198
Thr Leu Val Lys His Ser Gln Ser Glu Gln Val Pro Pro Cys Pro Ile	
40 45 50	
ggg atg agc cag ctg tgg gtg ggg tac agc tta ctg ttt gtg gag ggg	246
Gly Met Ser Gln Leu Trp Val Gly Tyr Ser Leu Leu Phe Val Glu Gly	
55 60 65	
caa gag aaa gcc cac aac cag gac ctg ggc ttt gct ggc tcc tgt ctg	294
Gln Glu Lys Ala His Asn Gln Asp Leu Gly Phe Ala Gly Ser Cys Leu	
70 75 80 85	
ccc cgc ttc agc acc atg ccc ttc atc tac tgc aac atc aac gag gtg	342
Pro Arg Phe Ser Thr Met Pro Phe Ile Tyr Cys Asn Ile Asn Glu Val	
90 95 100	
tgc cac tat gcc agg cgc aat gat aaa tct tac tgg ctc tcc act acc	390
Cys His Tyr Ala Arg Arg Asn Asp Lys Ser Tyr Trp Leu Ser Thr Thr	
105 110 115	
gcc cct atc ccc atg atg ccc gtc agc cag acc cag att ccc cag tac	438
Ala Pro Ile Pro Met Met Pro Val Ser Gln Thr Gln Ile Pro Gln Tyr	
120 125 130	
atc agc cgc tgc tct gtg tgt gag gca ccc tcg caa gcc att gct gtg	486
Ile Ser Arg Cys Ser Val Cys Glu Ala Pro Ser Gln Ala Ile Ala Val	
135 140 145	
cac agc cag gac atc acc atc ccg cag tgc ccc ctg ggc tgg cgc agc	534
His Ser Gln Asp Ile Thr Ile Pro Gln Cys Pro Leu Gly Trp Arg Ser	
150 155 160 165	
ctc tgg att ggg tac tct ttc ctc atg cac act gcc gct ggt gcc gag	582
Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr Ala Ala Gly Ala Glu	
170 175 180	
ggg gga ggc cag tcc ctg gtc tca cct ggc tcc tgc cta gag gac ttt	630
Gly Gly Gly Gln Ser Leu Val Ser Pro Gly Ser Cys Leu Glu Asp Phe	
185 190 195	
cgg gcc act cct ttc atc gaa tgc agt ggt gcc cga ggc acc tgc cac	678
Arg Ala Thr Pro Phe Ile Glu Cys Ser Gly Ala Arg Gly Thr Cys His	
200 205 210	
tac ttt gca aac aag tac agt ttc tgg ttg acc aca gtg gag gag agg	726
Tyr Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr Val Glu Glu Arg	
215 220 225	
cag cag ttt ggg gag ttg cct gtg tct gaa acg ctg aaa gct ggg cag	774
Gln Gln Phe Gly Glu Leu Pro Val Ser Glu Thr Leu Lys Ala Gly Gln	
230 235 240 245	
ctc cac act cga gtc agt cgc tgc cag gtg tgt atg aaa agc ctg	819

15

Leu His Thr Arg Val Ser Arg Cys Gln Val Cys Met Lys Ser Leu
 250 255 260

taggggtggca cctgccacgg gccctattct atagtgtcac ctaaatgcta gagctcgtg 879

atcagcctcg actgtgcctt c 900

<210> 12

<211> 260

<212> PRT

<213> Human

<400> 12

Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
 1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Lys Leu Ala Ser
 20 25 30

Met Arg Val Gly Tyr Thr Leu Val Lys His Ser Gln Ser Glu Gln Val
 35 40 45

Pro Pro Cys Pro Ile Gly Met Ser Gln Leu Trp Val Gly Tyr Ser Leu
 50 55 60

Leu Phe Val Glu Gly Gln Glu Lys Ala His Asn Gln Asp Leu Gly Phe
 65 70 75 80

Ala Gly Ser Cys Leu Pro Arg Phe Ser Thr Met Pro Phe Ile Tyr Cys
 85 90 95

Asn Ile Asn Glu Val Cys His Tyr Ala Arg Arg Asn Asp Lys Ser Tyr
 100 105 110

Trp Leu Ser Thr Thr Ala Pro Ile Pro Met Met Pro Val Ser Gln Thr
 115 120 125

Gln Ile Pro Gln Tyr Ile Ser Arg Cys Ser Val Cys Glu Ala Pro Ser
 130 135 140

Gln Ala Ile Ala Val His Ser Gln Asp Ile Thr Ile Pro Gln Cys Pro
 145 150 155 160

Leu Gly Trp Arg Ser Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr
 165 170 175

Ala Ala Gly Ala Glu Gly Gly Gly Gln Ser Leu Val Ser Pro Gly Ser
 180 185 190

Cys Leu Glu Asp Phe Arg Ala Thr Pro Phe Ile Glu Cys Ser Gly Ala
 195 200 205

Arg Gly Thr Cys His Tyr Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr
 210 215 220

Thr Val Glu Glu Arg Gln Gln Phe Gly Glu Leu Pro Val Ser Glu Thr

225

230

235

240

Leu Lys Ala Gly Gln Leu His Thr Arg Val Ser Arg Cys Gln Val Cys
245 250 255

Met Lys Ser Leu
260

INTERNATIONAL SEARCH REPORT

International Application No.

PCT/US 00/08678

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 A61K38/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the International search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE, CANCERLIT, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 856 184 A (SARRAS JR MICHAEL P ET AL) 5 January 1999 (1999-01-05) column 10, line 39 - column 11, line 12; claims 1-4; figures 7,8,10 column 12, line 14 - line 32	1-4,9-16
X	PRESTAYKO ET AL: "Type IV collagen domains inhibit adhesion and migration of tumor cells and block angiogenesis", PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH, US, PHILADELPHIA, PA: AACR, VOL. 39, PAGE(S) 45 XP002118641 abstract	1-4,9-16

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"Z" document member of the same patent family

Date of the actual completion of the international search

2 August 2000

Date of mailing of the international search report

22/08/2000

Name and mailing address of the ISA

European Patent Office, P.O. Box 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Noë, V

INTERNATIONAL SEARCH REPORT

Inter national Application No

PCT/US 00/08678

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 567 609 A (SARRAS JR MICHAEL P ET AL) 22 October 1996 (1996-10-22) abstract column 1, line 15 - line 24 column 4, line 15 - line 40 column 9, line 8 - line 19	1-12
X	KEFALIDES N A ET AL: "SUPPRESSION OF TUMOR CELL GROWTH BY TYPE IV COLLGEN AND A PEPTIDE FROL THE NC1 DOMAIN OF THE ALPAH3(IV) CHAIN" MEDICINA (BUENOS AIRES), vol. 59, no. 5-2, 1999, pages 553-553, XP002144122 the whole document	1,13
A	HAN JING ET AL: "A cell binding domain from the alpha-3 chain of type IV collagen inhibits proliferation of melanoma cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 33, 1997, pages 20395-20401, XP002144123 ISSN: 0021-9258 page 20400, column 2, paragraph 2; table 2	13-16
A	US 5 766 591 A (BROOKS PETER ET AL) 16 June 1998 (1998-06-16) abstract column 14, line 15 - line 23; examples 7,8,10	1,9,13
A	SETTY SUMAN ET AL: "Interactions of type IV collagen and its domains with human mesangial cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 20, 15 May 1998 (1998-05-15), pages 12244-12249, XP002144124 ISSN: 0021-9258 page 12245, column 1, paragraph 3	3,8,12, 16
P,X	WO 99 49885 A (UNIV KANSAS MEDICAL CENTER) 7 October 1999 (1999-10-07) the whole document	1-16
P,X	PETITCLERC E ET AL: "NEW FUNCTION FOR NON-COLLAGENOUS DOMAINS OF HUMAN COLLAGEN" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, 2000, pages 8051-8061, XP002144125 abstract page 8052, column 1, paragraph 2 page 8055 -page 8057; figures 4-6,8	1-4, 13-16

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-16 relate to an extremely large number of possible polypeptides. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the polypeptides claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to NC1 monomers of collagen type IV and their fragments.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 00/08678

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5856184 A	05-01-1999	US 5691182 A US 5567609 A AU 3000895 A EP 0767676 A WO 9600582 A	25-11-1997 22-10-1996 25-01-1996 16-04-1997 11-01-1996
US 5567609 A	22-10-1996	AU 3000895 A EP 0767676 A WO 9600582 A US 5691182 A US 5856184 A	25-01-1996 16-04-1997 11-01-1996 25-11-1997 05-01-1999
US 5766591 A	16-06-1998	US 5753230 A AU 709645 B AU 1995295 A CA 2184493 A CN 1151120 A CZ 9602711 A EP 0754059 A FI 963692 A HU 76086 A JP 10500398 T NO 963894 A SK 119096 A WO 9525543 A ZA 9502214 A	19-05-1998 02-09-1999 09-10-1995 28-09-1995 04-06-1997 14-01-1998 22-01-1997 18-09-1996 30-06-1997 13-01-1998 18-11-1996 09-07-1997 28-09-1995 08-02-1996
WO 9949885 A	07-10-1999	AU 3202599 A	18-10-1999